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Experimental basis for the prevention of breast cancer

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Abstract

Cancer chemoprevention involves intervention in the carcinogenic process by a natural or synthetic chemical that either blocks neoplasia development or arrests malignant phenotype progression. The chemopreventive test agent must experimentally be established as safe before a clinical trial. In our laboratory, inhibition of carcinogen-induced development of precancerous lesions in the mouse mammary gland organ culture model is used as a primary screen to select chemopreventive agents for *in vivo* efficacy evaluation. A nearly 75% correlation apparently exists between the efficacy observed *in vitro* and *in vivo* carcinogenesis. For *in vivo* experiments, MNU- and DMBA-induced mammary tumours in rats are the models of choice. Numerous agents have been identified and progressed to preclinical toxicity and clinical trials. More recently, combination chemoprevention has received considerable attention, since no known chemopreventive agent sufficiently reduces tumour incidence in rats. The sequence of events for establishing the experimental basis for chemoprevention of breast cancer is described.

Keywords: Organ culture; Mammary lesions; Carcinogenesis; Chemopreventive agents; Combination chemoprevention

1. Introduction

The ultimate overall objective of cancer chemoprevention research is to advance knowledge in identifying and characterising entities that might reduce the risk of the human population developing cancer. In theory, cancer chemoprevention can be defined as an intervention in the carcinogenic process by a chemical that either blocks neoplastic process induction or prevents transformed cells from progressing to malignant phenotype. It also may encompass a reversal of the process of progression [1,2]. Ideally, eliminating carcinogens from the environment would reduce carcinogenesis; however, this is not feasible. In practice, a putative intervention agent must be introduced that might enhance the physiological processes protecting humans against preneoplastic cell progression or neoplastic cell growth. The basic well defined strategy for developing chemopreventive agents includes evaluation of (1) potentially effective agents as determined from epidemiological results and experimental studies, (2) preclinical efficacy of candidate agents, and (3) preclinical and clinical safety of newly identified agents [3].

The experimental basis of chemoprevention involves identifying and characterising new agents using a variety of *in vitro* and *in vivo* models. Once the efficacy of a newly identified chemopreventive agent is established, its tolerance, toxicity, stage specificity and mechanism of action should be determined [4]. The mechanistic studies allow for design of analogues that are more active, less toxic and of enhanced chemopreventive value. The activity of a potential chemopreventive agent can be judged by using a three-tier model approach. (1) Screening potentially useful chemopreventive agents using *in vitro* models [5]. These include cell culture and organ culture models. The approach would be to determine if the chemopreventive test agent prevents cell transformation induced by a carcinogen or prevents growth of transformed cells. (2) Analysing specific biomarkers modified by chemopreventive agents in tissue culture and in short-term *in vivo* models. This provides a rapid assay for predicting the responsiveness of test agents [6]. (3) More confirmatory assays to determine the efficacy of compounds in animal models [4,7]. This allows one to evaluate the effectiveness of a chemopreventive agent in target organ-specific chemically-induced carcinogenesis models.

As shown in Fig. 1, normal cells can be transformed chemically. The initiated cells can then be promoted to

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express a preneoplastic phenotype by either endogenous or exogenous promotional agents. These cells, in the appropriate environment, can cause neoplastic cells. The neoplastic cells can form tumours and progress to more aggressive tumours. Based on their mode of action, new chemopreventive agents can be designed to interfere at any of these stages.

1.1. Target organ specificity

Traditionally, experimental models are used to predict sensitivity to a chemopreventive agent in human clinical trials. The experimental models have included both *in vitro* and *in vivo* evaluations. In the past, if a compound proved active in a given experimental model, it was believed to serve as a possible chemopreventive agent, possibly for most sites. However, with the accumulation of new studies in the literature, it has become apparent that such a universal relationship does not exist. For example, certain retinoids (such as 13-*cis* retinoic acid) are effective against experimental skin and urinary bladder carcinogenesis but ineffective against chemically-induced mammary carcinogenesis. Similarly, piroxicam, which is highly effective against colon carcinogenesis in rodents, is ineffective against mammary carcinogenesis. N-[4-hydroxyphenyl]retinamide (4-HPR) is highly effective against mammary carcinogenesis but is ineffective against chemically induced oesophageal and colon carcinogenesis [4]. This raises an important concern about the appropriate model systems for predicting the response to a given chemopreventive agent. Thus, for evaluating the efficacy of chemopreventive agents against breast cancer prevention, chemically-induced mammary carcinogenesis models may serve better than experimental carcinogenesis models of other organs. Likewise, for predicting the response of a chemopreventive agent against experimental mammary carcinogenesis *in vivo*, appropriate mammary cell culture or organ culture models are better suited than cell and organ cultures of other organs. Therefore, in our laboratory, we concentrate on evaluating mammary cells and organ cultures for predicting responsiveness of chemopreventive agents in chemically-induced mammary carcinogenesis.

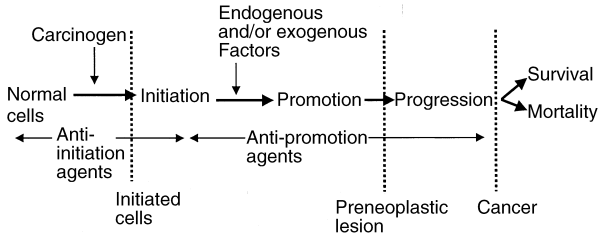

Fig. 1. Schematic diagram to show stages in mammary carcinogenesis and efficacy of chemoprevention at various stages.

2. Materials and methods

The experimental approach to evaluating chemoprevention of breast cancer in our laboratory included mammary gland organ culture and chemically-induced mammary carcinogenesis.

2.1. Mammary gland organ culture (MMOC)

Mammary glands from young virgin female mice respond dramatically to hormones in serum-free medium. This physiologically similar response to hormones was further characterised to determine if the glands would respond to chemical carcinogens. When the glands were exposed to chemical carcinogen 7, 12-dimethylbenz(a)anthracene (DMBA) for 24 h in culture, they developed hyperplastic alveolar nodule-like mammary lesions under appropriate hormonal conditions [8]. The epithelial cells prepared from these lesions form mammary adenocarcinoma when transplanted into syngeneic mice [9]. We have termed these lesions as mammary alveolar lesions (MAL) and taken advantage of this *in vitro* model to evaluate the efficacy of potential chemopreventive agents. The entire culture procedure has been previously described in detail [10,11] and is schematically presented in Fig. 2. More recently, we modified the organ culture system and replaced aldosterone and hydrocortisone for oestradiol and progesterone. Under these experimental conditions, mammary ductal lesions (MDL) are formed. MDL resemble the

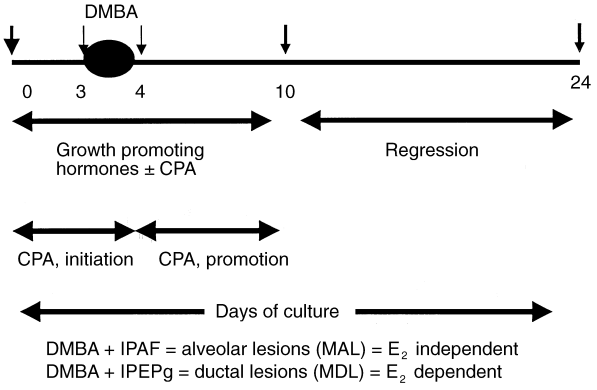

Fig. 2. Experimental design to evaluate chemopreventive agent in MMOC. During a 24-day culture, mammary glands are exposed to DMBA on day 3 for 24 h in the presence of growth promoting hormones. The hormones are withdrawn after 10 days of culture for additional 14 days. The MAL incidence is determined in stained glands. The chemopreventive agent is included in the medium for the first 10 days of culture. For evaluating stage-specific effects on initiation and promotion, the agent is included in the medium for either 0 to +4 days for initiation or +4 to +10 for promotion-specific determination of chemopreventive agent's activity. Oestrogen-dependent mammary ductal lesions (MDL) are formed when oestrogen and progesterone (IPEPg) are present in the growth medium whereas aldosterone and hydrocortisone (IPAF) are present for alveolar lesions (MAL).

ductal carcinoma *in situ* (DCIS) of human. This model has been employed to evaluate chemopreventive agents with anti-oestrogenic properties.

2.2. Evaluation of chemopreventive agents in MMOC for their selective role in initiation/promotion or both

As shown in Fig. 1, the action of a chemopreventive agent can be stage-specific. The agent may be effective as an anti-initiator, anti-promoter or both. This distinction is often useful to hypothesise a possible mechanism of action of a chemopreventive agent. We have extended the MMOC model to incorporate evaluation of chemopreventive agents for their efficacy as stage-specific agents by including 25 ng/ml 9,10-tetradecanoylphorbol ester (TPA) between days 9–14 of the culture [12]. As shown in Fig. 2, the chemopreventive agent is included in the medium either before and during the carcinogen treatment or after the carcinogen is removed between days 4–10. Results are compared with the conventional culture protocol. This allows one to distinguish between possible anti-initiation or anti-promotion activity of the compound.

2.3. *Chemically-induced mammary carcinogenesis* in vivo

Significant effort has been directed towards developing new animal tumour models and modifying existing ones to provide better model systems for chemoprevention studies. In order to establish experimental models for carcinogenesis of mammary glands or any other organ, the following goals must be met. (1) The tumours produced have histological and biological similarities to human lesions. (2) The resulting tumours have to be induced with a single dose (or at most only a few carcinogenic doses). (3) The tumour induction has to be specific for the target organ of interest and should not be compromised by induction of tumours at several sites.

The most widely used rat mammary carcinogenesis models include polycyclic hydrocarbons, such as DMBA- or benzo(a)pyrene-induced or direct-acting carcinogen N-methyl-N-nitrosourea (MNU)-induced mammary cancers in Sprague–Dawley female rats. Other carcinogens and other rat species such as Fisher or Wistar Furth rats have been studied as possible animal models to induce mammary cancers [13]. However, due to poor incidence and reduced specificity, these models have not been frequently employed.

DMBA- and MNU-induced mammary carcinogenesis models are similar in many respects. Both carcinogens induce mammary adenocarcinoma with a single dose. The tumour induction is mammary gland-specific. There is well-established dose-dependence, and tumours are developed without any systemic toxicity. Both models are extremely reproducible. The multiplicity can also be adjusted with carcinogen dose. Basic differences, however, exist. For example, DMBA requires metabolic activation; thus, the model is more suited to studying initiation and promotion or for evaluating effects of agents that may affect parameters of carcinogen metabolism and activation. MNU, on the other hand, is a direct-acting carcinogen and is not well suited to studying stage-specific activity of a chemopreventive agent. MNU-induced mammary tumours are largely adenocarcinomas, whereas tumours induced by DMBA consist of 60% adenocarcinomas and 40% benign fibroadenomas [14,15]. Thus, DMBA-induced tumours require histopathology for confirming the carcinoma incidence. Finally, unlike DMBA, MNU-induced tumours are locally invasive and metastasise to distant sites.

Experimentally, Sprague–Dawley female rats 50 days old are used for chemoprevention of mammary carcinogenesis. For MNU-induced carcinogenesis experiments, rats are injected intravenously (i.v.) in the jugular vein with 50 mg/kg-body weight of MNU in acidified saline (pH 5.0) solution. Alternatively, MNU can be administered subcutaneously (s.c.) [16] or intraperitoneally (i.p.) [17] at the same dose level with similar results. For DMBA, the carcinogen is dissolved in sesame oil at a dosing concentration of 12–20 mg in 1 ml. The animals are fasted for 18 h prior to carcinogen treatment. They receive an intragastric dose of 12–20 mg DMBA by intubation. After carcinogen administration, the animals are randomised into groups maintaining average weight to be constant in all groups. With both carcinogens, 100% of the animals develop mammary tumours by 3–4 months. Tumours of other sites are rare. At necropsy, animals are sacrificed by CO_2 asphyxiation, tumours and organs of interest are removed, a small portion is fixed in 10% formalin for histopathological evaluation, and the rest is frozen in liquid nitrogen for biochemical analysis.

2.4. *Chemoprevention experiments* in vivo

One of the primary requirements for chemoprevention studies is that the chemopreventive agent has to be non-toxic. This is true since the overall objective of chemoprevention research is to employ a chemopreventive agent either in a healthy population or in women at a higher risk of developing breast cancer who are otherwise healthy [1]. Thus, before conducting a chemoprevention study, a dose tolerance experiment is usually performed. This allows determination of the maximum concentration of chemopreventive agent tolerated by rats without any symptoms of toxicity. This maximum tolerated dose (MTD), serves as a guideline for the safe dose to be used in a carcinogenesis protocol [18].

Typically, determination of MTD is carried out using a range of five to six concentrations. The initial concentration for a given chemopreventive agent is selected from the literature or from the data sheet provided by the supplier. Sprague–Dawley rats of the same age as those in the carcinogenesis experiments are used for the study. Animals are distributed into various treatment groups, with each group receiving increasing concentrations of the chemopreventive agent. The agent is mixed with semipurified-powdered diet and fed to the animals *ad lib*. Animals are monitored twice a day for general health and weighed once weekly. After 6 weeks, animals in all groups are sacrificed except in the dose group selected for the chemoprevention study. This serves as a '6-week lead time' for assessing cumulative toxicity of the chemopreventive agent being studied in chemoprevention.

Experimental chemoprevention of mammary carcinogenesis is typically evaluated at 40 and 80% of the MTD levels of chemopreventive agents [19]. The experimental design is schematically described in Fig. 3. As described earlier, animals are randomised according to body weight and treated with carcinogen at 50 days old. Animals receive experimental diets beginning 2 weeks before carcinogen treatment and continued to the end of the study (−2 to end), or from 2 weeks before carcinogen treatment to 1 week post carcinogen treatment (−2 to +1 weeks), or 1 week after carcinogen treatment to the end of the study (+1 to end) [20]. These treatments, respectively, encompass the entire carcinogenesis period during the initiation or promotion period of carcinogenesis. Although both MNU and DMBA are interchangeably used to evaluate stage-specific effects of compounds on carcinogenesis, DMBA is more suited for determining the effects of a chemopreventive agent on initiation. This is because DMBA needs to be metabolised to an active carcinogen, and an anti-initiator (either an antioxidant or agents that prevent metabolism of carcinogen) can be identified. In contrast, MNU causes point mutations, and a true anti-initiator often can not prevent mutation. However, anti-promotional agents can be identified with either carcinogen.

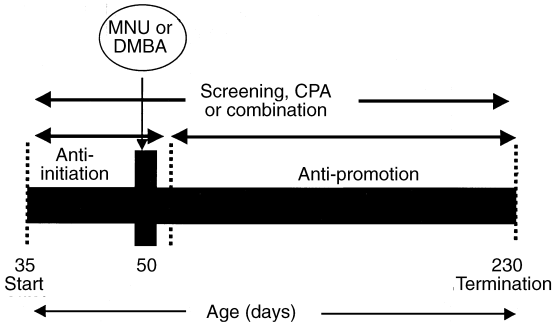

Fig. 3. Schematic diagram to show chemoprevention of mammary carcinogenesis *in vivo*. Typically, female Sprague–Dawley rats receive a single intravenous injection of MNU or intragastric intubation of DMBA at 50 days old. For screening a chemopreventive agent, the test chemopreventive agent is administered beginning 2 weeks prior to carcinogen treatment on day 35 and continued until the end of the study. However, for the distinction between the specific effects during either initiation or promotion phases the chemopreventive agent is administered for a shorter duration as shown in the figure.

Rats are palpated once a week for the presence of mammary tumours and weighed once a week to assure that the compound does not cause any adverse effects. The incidence of adenocarcinoma is determined by histopathological evaluations, and the multiplicity is determined by calculations of the number of tumours per rat. Results are statistically evaluated for significance. Tissues are saved for mechanistic studies.

2.5. Combination chemoprevention

Most single-agent chemoprevention experiments in animals models have resulted in the selection of several candidate chemopreventive agents for preclinical toxicity studies, clinical trials to evaluate toxicity in humans and actual chemoprevention trials for women at high risk of developing breast cancer [4,21]. The experimental approach is similar to that of chemoprevention with a single agent. However, determination of doses of combination agents requires an extensive dose tolerance study to ensure that the combination is not inducing any toxicity. In our laboratory, we normally select 50% MTD of both agents as a starting combination dose and increase the dose of one chemopreventive agent, keeping one agent at the 50% MTD level. Once doses are selected, the experimental protocol for the chemoprevention study remains similar to that of a single agent. In addition to combining two chemopreventive agents for enhancing efficacy, combining a chemopreventive agent and depriving hormones by ovariectomy has also been investigated [22].

3. Results and discussion

Intense effort has been directed in recent years toward identifying effective chemopreventive agents for humans. It is necessary to establish the safety of a chemopreventive agent for human use before its application. Therefore, an experimental basis for establishing the efficacy of chemopreventive agents has to be recognised. In this report, I have summarised a step-wise strategy, recognised by the National Cancer Institute and adopted in our laboratory, for evaluating chemopreventive agents under experimental conditions prior to preclinical toxicity and clinical trials for breast cancer patients.

3.1. Cell culture models

Ideally, cell culture models would be useful in studying the effects of chemopreventive agents, since they

represent a homogenous cell population and the effects of a given growth modulator can be studied in a controlled environment. However, epithelial cells from normal mammary glands can not be propagated for more than a few passages. Also, one truly needs to study the effects of a potential chemopreventive agent during cell transformation. None of the cell culture systems currently available provides this [23]. Several studies have used HBL-100 human breast epithelial cells to study the effects of chemopreventive agents such as retinoids and vitamin D analogues [24]. The 'normal-like' cells do not form cancer but are transformed with SV40 virus. Since SV40 interferes with many endogenous receptors, including vitamin D receptors (VDR), vitamin D analogues are inactive against HBL 100 cells [24]. Similarly, 4-HPR, a retinoid analogue, has also been ineffective against HBL-100 cells. Another cell culture model using preneoplastic human mammary epithelial cells (184-B5/HER cells derived from reduction mammoplasty and transformed by overexpressing *HER-2/neu* oncogene) has been used to evaluate the effects of retinoids and several other natural products [25]. Results on the effects of 4-HPR in this model showed that incubation of these cells with 400 nM 4-HPR for 14 days resulted in an 80% inhibition in colony formation on soft agar. The effects were reversible if the cells were incubated with the retinoid for 7 days rather than 21 days. Several other human breast epithelial cells and rodent mammary epithelial cells have been maintained in culture, including cultures of mammary epithelial cells on plastic, on collagen gel on floating gel, and on reconstituted basement membrane. All culture systems offer advantages and disadvantages. However, they have not been used for chemoprevention studies [23].

3.2. Organ culture model

As described earlier, a short exposure of the gland to DMBA results in the development of either alveolar or ductal precancerous lesions in serum-free medium. We have taken advantage of this cell transformation model to evaluate the effects of known and potential chemopreventive agents. During the past 10 years, we have evaluated more than 200 potential chemopreventive agents for their effectiveness in the organ culture model. In a typical experiment, DMBA normally induces 60–80% incidence of MAL. In groups of 15 glands, a greater than 60% reduction in the incidence of MAL by a chemopreventive agent turns out to be statistically significant. Some examples of the known agents are listed in Table 1 to show their effectiveness in MMOC. Results show that some agents (such as piroxicam and sulphasalazine) may be highly effective against colon carcinogenesis but ineffective in MMOC; whereas agents such as 4-HPR [15], 1α-hydroxyvitamin D5 [26], resveratrol [27], brassinin [28], deguelin [29] and troglitazone [30] are effective in MMOC. These results suggest a distinct target organ-specificity and that MMOC is an excellent model for predicting the responsiveness of a chemopreventive agent *in vivo*. Thus far, there appears to be a 75% correlation between MMOC and *in vivo* carcinogenesis.

3.3. *Mammary carcinogenesis* in vivo

As described in previous sections, DMBA and MNU induce mammary cancers selectively and, therefore, are ideal to serve as the experimental basis for development of chemopreventive agents for human use. Very early chemoprevention studies by Moon and coworkers formed a basis for evaluating certain parameters to judge the effectiveness of chemopreventive agents in mammary carcinogenesis [4,31,32]. These include the time of carcinogen instillation, dose and routes of carcinogen administration, evaluation of efficacy of an agent on the inhibition of tumour incidence, multiplicity and increased latency. The group evaluated more than 70 potential chemopreventive agents using either MNU-

Table 1
Examples of the correlation of effectiveness of chemopreventive agents in MMOC and experimental rat mammary carcinogenesis[a]

Agents effective in MMOC	Effectiveness in experimental rat mammary carcinogenesis	Agents ineffective in MMOC	Effectiveness in experimental rat mammary carcinogenesis
4-HPR	Effective	Piroxicam	Ineffective
1α-Hydroxyvitamin D5	Effective	Diallyl disulphide	Ineffective
Oltipraz	Effective	Morin	Ineffective
Tamoxifen	Effective	N-Butyric acid	Ineffective
9-cis-Retinoic acid	Effective	Sodium Thiosulphate	Ineffective
DHEA	Effective	Retinyl acetate	Effective
DFMO	Effective	Melatonin	Effective
Carbenoxolone	Effective	Sarcophytal A	Ineffective
Indomethacin	Effective		
Ellagic acid	Ineffective		
Deguelin	Effective		
Thiolutin	Ineffective		

[a] DHEA, dehydroepiandrosterone; DFMO, D,L-alpha-difluoromethylorthinine.

or DMBA-induced mammary carcinogenesis models. The retinoid analogue 4-HPR was extensively studied in our laboratory either as a single agent or in combination with other chemopreventive agents and was selected for further development for human clinical trials. Similarly, 1α-hydroxyvitamin D_5 was found to be active *in vitro* in MMOC, and we recently observed that it suppresses MNU-induced mammary cancer incidence and multiplicity in a dose-dependent fashion in rats (data not shown). The agent is currently being evaluated for preclinical toxicity in rats and dogs under good laboratory practice protocols in our laboratories.

In addition to conventional long-term mammary carcinogenesis models, a rapid MNU-induced mammary carcinogenesis model was developed by Thompson and coworkers [33]. In this model, rats receive the carcinogen intraperitoneally at 28–35 days old rather than 50 days old. This allows a rapid development of tumours and early lesions. Thompson and his group also defined a timetable that can accurately predict the development of early localised intraductal carcinoma that have not yet progressed to form invasive carcinoma. This model has been successfully adopted to evaluate the effects of an active selenium analogue: methylselenocysteine [34]. This model is relatively new and has not been extensively used to evaluate the efficacy of chemopreventive agents.

3.4. Combination chemoprevention

No chemopreventive agent to date has shown total suppression of chemically-induced carcinogenesis. The nearest total prevention of mammary carcinogenesis is achieved by ovariectomy. However, ovariectomy as a preventive mode for otherwise healthy women is not possible; therefore, the most logical alternative approach is to combine two chemopreventive agents (again at non-toxic safe doses) to enhance protection from that obtained with a single agent. The strategy involves combining two active agents with different mechanisms of action so that the chemopreventive action may be mediated more effectively. Alternatively, the combination of two agents could be selected so that the second agent can reduce the toxicity of an active chemopreventive agent, allowing an increased dose of the latter chemopreventive agent. Such combination chemoprevention has been specifically exemplified in mammary carcinogenesis models (Table 2).

Combination chemoprevention in *in vitro* models has not received much attention. Only recently have we initiated an evaluation of combining agents in MMOC. In one study, we observed that a peroxisome proliferator-activated receptor γ (PPARγ) ligand, troglitazone, is moderately effective in suppressing MAL in mice, whereas a specific ligand of the retinoid X receptor (RXR) was ineffective. However, a combination of these two ligands inhibited MAL development by nearly 100% [30]. This model provides an experimental basis for logical combinations that may eventually be evaluated in *in vivo* models. Several investigators (including studies from our laboratory) have shown combinations of chemopreventive agents as being more efficacious than single agents. Some of these agent combinations include 4-HPR and tamoxifen, retinyl acetate and ovariectomy, retinyl acetate and tamoxifen, retinyl acetate and 2-bromo-ergocryptin, 4-HPR and calcium glucarate and retinyl acetate and selenium. More recently, combination of a non-toxic analogue of vitamin D (RO24-5531) and tamoxifen was shown to be more effective than the vitamin D analogue alone. In addition, a PPARγ-ligand and tamoxifen combination shows enhanced protection against MNU-induced mammary carcinogenesis compared with either agent alone [35]. These are only a few examples of the many combinations evaluated in chemical carcinogenesis of the mammary gland. Many of them have provided additive or synergistic effects, though some have not been successful. Some of the failed effects of combination agents include a combination of a retinoid with a maleic anhydride-divinyl ether copolymer [36] and

Table 2
Effectiveness of combination chemoprevention in experimental mammary carcinogenesis[a]

Experiment	Combination	Carcinogen	Comments	[Ref.]
1	4-HPR + ovariectomy	MNU, DMBA	Synergistic	[22]
2	4-HPR + tamoxifen	MNU, DMBA	Additive	[4,38]
3	9-cis-Retinoic acid + tamoxifen	MNU	Enhanced	[39]
4	Progesterone, RU16117, aminoglutathamide and toremefene	MNU	No benefit of combinations	[37]
5	Diallyl disulphide + Se	DMBA	Enhanced	[40]
6	Ellagic acid + Se	DMBA	Enhanced	[40]
7	Quercetin + Se	DMBA	Enhanced	[40]
8	RO24-5531 + tamoxifen	MNU	Enhanced	[41]
9	13-*cis* Retinoic acid + calcium glucarate	MNU	Combination of ineffective doses	[42]
10	Miso + tamoxifen	DMBA	Enhanced	[43]
11	Melatonin + tamoxifen	DMBA	Ineffective	[44]

[a] Se, selenium; R024-5531, vitamin D analogue; MNU, N-methyl-N-nitrosourea; DMBA, 7,12-dimethylbenz(a)anthracene.

various combinations of tamoxifen, α-tocopherol, aminoglutethimide, ergocryptin and selenium [37]. In this study, neither single agents nor combinations were effective. None the less, there is a clear notion that combined chemopreventive agents may prove more valuable than single agents.

3.5. Experimental basis for other modifying factors for mammary carcinogenesis

Besides conducting *in vitro* and *in vivo* studies with new chemopreventive agents prior to their preclinical toxicity trials and possible clinical trials, two other major categories must be mentioned. Although in-depth description of this section is clearly beyond the scope of this article, the importance of chemopreventive agent metabolism and its mode of action are extremely important. The litmus test for an outstanding chemopreventive agent is that it should be absolutely safe, highly efficacious, effective at very low-doses and work well in combination with other agents. The metabolism and mechanistic studies provide an effective design for the synthesis of congeners of active chemopreventive agents.

In summary, this article makes a compelling case for the necessity of employing *in vitro* and *in vivo* models as experimental bases of breast cancer chemoprevention in humans. Some models are better suited to evaluating the effects of new chemopreventive agents; however, in general, a well-designed stepwise procedure ensures proper selection of agents for preclinical toxicity studies and clinical trials as a next step.

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