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Original Paper

3,3′,4,4′-Tetrachlorobiphenyl (TCB) can Enhance DMBA-induced Mammary Carcinogenesis in the Rat

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Polychlorinated biphenyls (PCBs) are fat-soluble environmental pollutants which can be stored in the fatty tissue of breast and secreted in milk. Previous studies have shown that PCBs can influence liver carcinogenesis in animal models but no such studies have been reported in breast. These experiments aimed to determine whether a PCB congener could influence mammary carcinogenesis using the rat DMBA-induced mammary tumour model system. 3,3′,4,4′-Tetrachlorobiphenyl (TCB) enhanced the development of DMBA-induced mammary tumours in young female rats and did so in animals fed either a low-fat (5% w/w corn oil) or a high-fat (20% w/w corn oil) diet. The combination of TCB and high-fat diet resulted in tumours growing so fast that the experiment had to be terminated at 10.5 weeks for humane reasons. At termination the total numbers of tumours in each group of 20 rats were: 4 in the low-fat group, 22 in the low-fat plus TCB group, 25 in the high-fat group and 50 in the high-fat plus TCB group. Histopathological analysis confirmed that 98% of the tumours were mammary carcinomas, predominantly *in situ* ductal carcinomas, but, in addition, revealed that 13 of the tumours had an invasive phenotype of which 12/13 had all arisen in TCB-treated animals. This demonstrates, for the first time, that a PCB congener can influence mammary carcinogenesis. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: polychlorinated biphenyls, DMBA, mammary carcinogenesis
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INTRODUCTION

POLYCHLORINATED BIPHENYLS (PCBs) are one of the most widespread, persistent man-made chemicals in the ecosystem giving rise to serious environmental contamination [1–3]. The fat-soluble nature of PCBs results in their retention in fatty tissues of humans [3, 4] and poses a potential hazard to health. Since PCBs are found in fatty tissue of breast and can be secreted in human milk [3, 4], this poses a question as to a potential role in breast cancer. There are some reports that breast fat and serum lipids of women with breast cancer contain higher levels of some organochlorine residues than non-cancer controls [5–11], but a formal link between PCBs and breast cancer is unproven, particularly since high occupational exposure to PCB has not been shown to increase breast cancer incidence [12].

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The major target site for PCB toxicity in the body appears to be the liver and studies of PCBs in carcinogenesis report the development of tumours mainly in the liver [3, 4]. In view of the ability of PCBs to persist in breast fat and to be secreted in breast milk, the experiments reported here were designed to determine whether one PCB congener with high toxicity and high retention in body fat 3,3′,4,4′-tetrachlorobiphenyl (TCB) [3] could influence development of mammary tumours.

One well-established model system for the study of mammary tumour development involves the use of a single oral dose of 7,12-dimethylbenz(α)anthracene (DMBA) to initiate mammary cancer in young rats [13] and interacting components can be studied for their ability to alter growth of the tumours [14, 15]. Various components have been shown over the years to influence tumour development in this model system including age, endocrine status, composition of the diet and dose of DMBA [14, 15]. In terms of the diet, fat has

been shown to be important since rodents fed high-fat diets develop more DMBA-induced mammary tumours faster than control animals fed low-fat diets [16–20]. The aim of this experiment was, therefore, to determine whether the inclusion of TCB with DMBA in the single initial dose could enhance mammary tumour incidence or tumour number in rats. However, in view of both the influence of level of dietary fat in this model system and the fat-soluble nature of PCBs, studies were carried out using both high- and low-fat diets.

MATERIALS AND METHODS

Animals

Sprague–Dawley (SD) virgin female rats were purchased at 45 days of age (Charles River, Kent, U.K.) and housed two animals to a cage, in a controlled environment with a 12 h light/12 h dark cycle. Food and water was available *ad libitum* during the course of the experiment. The experiment was carried out under U.K. licence adhering strictly to the U.K. Coordinating Committee on Cancer Research (UKCCCR) guidelines for the welfare of animals used in experimental neoplasia.

Diets

Rats were fed semipurified diets with either a low-fat (5% (w/w) corn oil) or high-fat (20% (w/w) corn oil) composition. All diets were prepared in 3 kg lots and stored at 4°C until use. Low-fat diets contained w/w the following ingredients: 28% casein, 55% dextrose, 5% corn oil, 6% cellulose, 1% vitamin mix (AIN 76), 4.5% salt mix (AIN 76), 0.3% methionine, 0.2% choline bitartrate. High-fat diets contained the same ingredients but differed in the proportion of dextrose (40%) and corn oil (20%).

Experimental design

Mammary tumours were initiated in 80 rats at 50 days of age by intragastric administration of a single dose of 10 mg of dimethylbenz(α)anthracene (DMBA) in 0.5 ml of corn oil. 40 rats were also given 10 mg/kg body weight of 3,3′,4,4′-tetrachlorobiphenyl (TCB) by intragastric administration at the same time as the DMBA and were subsequently also fed for one week a further 500 μg of TCB per gram of corn oil in their diet. Of the 40 rats given TCB, 20 rats were then fed the low-fat diet and 20 rats were fed the high-fat diet. Similarly, of the 40 rats receiving no TCB, 20 rats were also fed the low-fat diet and 20 rats were fed the high-fat diet. All rats were inspected daily, but were weighed and palpated for mammary tumours at weekly intervals. The experiment was prematurely terminated at 10.5 weeks for reasons of humane treatment of animals.

At autopsy, animals were opened by midline incision from the pubis to the submaxillary area and examined for the existence of palpable and non-palpable tumours. All grossly visible tumour nodules were counted, removed, measured and transferred into formalin in individual containers. Tissues were processed routinely and finally embedded in paraffin wax. Sections of 3 μm thickness were cut and stained with haematoxylin and eosin. Histological features of the tumours were analysed independently by two histopathologists (MS, TK) and tumours were classified according to the criteria laid down previously for rat mammary tumours [21].

Liver from each rat was also collected into formalin in an individual container and processed in the same way as the tumours.

Statistical analysis

Data were analysed using the odds-ratio programme in the standard statistical application software (SAS) package.

RESULTS

The time course of appearance of DMBA-induced mammary tumours in each of the 4 groups of 20 rats is shown in Figure 1. The total number of tumours per group was increased by the presence of TCB for rats fed either a low-fat or a high-fat diet. Statistical analysis showed that the differences in tumour load between the low-fat and the low-fat plus TCB groups were highly significant at 8 weeks ($P = 0.0006$), 9 weeks ($P = 0.0001$) and 10 weeks ($P = 0.0001$). A similar result was obtained when comparing the high-fat with the high-fat plus TCB groups at 8, 9 and 10 weeks ($P < 0.005$). Control rats fed a low-fat diet alone had the lowest tumour development, with the first tumour appearing only after 8 weeks and a total of only 4 tumours at 10.5 weeks. Treatment with TCB on a low-fat diet resulted in appearance of the first tumour after 6 weeks and a total of 22 tumours at 10.5 weeks. The high-fat diet alone resulted in appearance of the first tumour after 4 weeks and a total of 25 tumours after 10.5 weeks. Treatment with TCB and a high-fat diet resulted in even earlier appearance of the first tumour after 3 weeks and a total of 50 tumours at 10.5 weeks. The numerous, fast-growing mammary tumours in the rats given TCB whilst on a high-fat diet necessitated termination of the experiment at 10.5 weeks for humane reasons.

Figure 2 shows the time course of DMBA-induced mammary tumour appearance expressed as percentage of the 20 rats developing tumours. The risk of a rat developing a tumour appeared to increase when TCB was administered. Statistical comparison of the low-fat with the low-fat plus TCB group showed the increase in tumour incidence to be highly significant from the ninth week of the experiment ($P = 0.05$), at 10 weeks ($P = 0.01$) and at autopsy ($P = 0.005$). Comparison of the high-fat with the high-fat plus TCB group

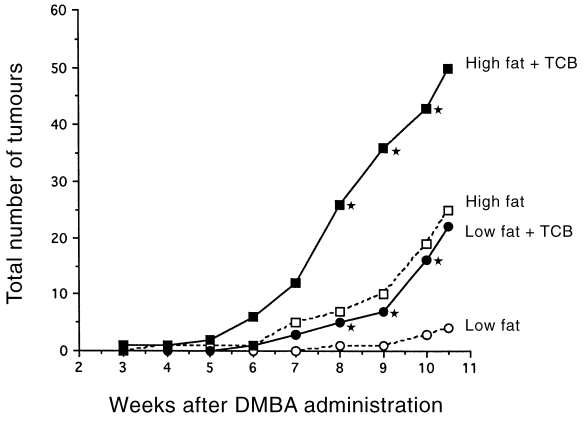

Figure 1. Effect of 3,3′,4,4′-tetrachlorobiphenyl (TCB) in conjunction with either a low-fat or a high-fat diet on DMBA-induced mammary tumour load. Each point represents the total number of palpable mammary tumours in a group of 20 rats treated identically and is presented as a function of time after DMBA administration. Treatment groups ($n = 20$) were low-fat diet alone, high-fat diet alone, low-fat diet with TCB administration, high-fat diet with TCB administration as indicated. *Significant difference ($P < 0.005$) between pairs of points with or without TCB at the same time and same fat level.

showed a significant increase in tumour incidence with TCB at 8 weeks ($P = 0.03$), but there was no significant difference at autopsy. Comparison of the low-fat plus TCB with the high-fat plus TCB-group showed a significant increase in tumour incidence in the higher fat at 8 weeks ($P = 0.03$) but not at subsequent time points.

Average initial whole body weights (± SEM) of each group of 20 rats were similar (194.4 ± 2.3 g low-fat group; 194.4 ± 1.8 g high-fat group; 193.7 ± 2.0 g low-fat plus TCB group; 195.8 ± 1.7 g high-fat plus TCB group). Measurement of final whole body weights confirmed that the high-fat diet had increased the body weight of the rats over the 10.5 weeks (322.0 ± 4.8 g low-fat group; 347.2 ± 6.6 g high-fat group; 315.2 ± 3.9 g low-fat plus TCB group; 345.0 ± 6.3 g high-fat plus TCB group). However, administration of TCB did not affect whole body weights, since there were no obvious differences in weight gain between the low-fat and low-fat plus TCB groups nor between the high-fat and high-fat plus TCB groups.

Histopathological examination of the mammary tumours in all 80 rats confirmed that all of the tumours except two were of mammary ductal epithelial origin and were of a malignant phenotype. This is in line with previous publications using this model system [14, 15]. Tumours were analysed and classified according to previously published criteria [21] and showed mainly cribriform morphology or cribriform mixed with other histologies. Of particular interest, however, was the finding of invasive characteristics in 13 of the tumours and that 12/13 of the tumours had all arisen in animals treated with TCB (5 at low-fat and 7 at high-fat). Figure 3 shows the histopathological appearance of two invasive carcinomas, each from a different rat.

At autopsy, the liver was investigated in all the rats. No nodules were detected in any of the 80 rats used. Histopathological analysis of all 80 livers showed mild microvesicular and macrovesicular fatty changes but there was no evidence for any hyperplasia in any of the livers (data not shown).

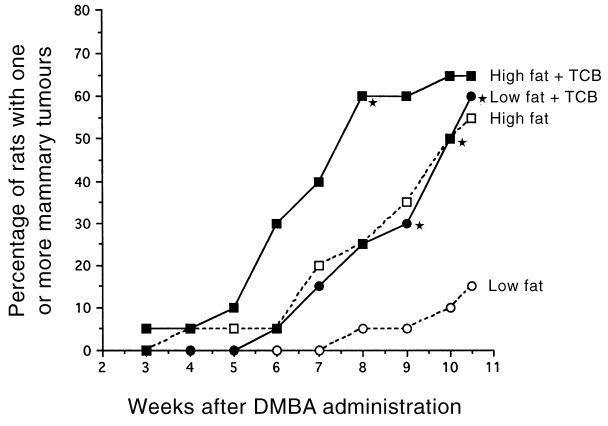

Figure 2. Effect of 3,3′,4,4′-tetrachlorobiphenyl (TCB) in conjunction with either a low-fat or a high-fat diet on DMBA-induced mammary tumour latency. The percentage of rats developing palpable mammary tumours as a function of time after DMBA administration is given for control rats on a low-fat diet or a high-fat diet and for experimental rats given TCB and then fed on a low-fat diet or a high fat diet ($n = 20$ for each treatment). *Significant difference ($P < 0.05$) between pairs of points with or without TCB at the same time and same fat level.

DISCUSSION

This study has demonstrated that TCB can enhance the development of DMBA-induced mammary tumours in the rat. Histopathological analysis confirmed that 98% of the tumours were ductal mammary carcinomas as expected [14, 15]. However, given the usually benign histological features of DMBA-induced rat mammary tumours, it was surprising to find that 13% of the tumours in this experiment had characteristics of an invasive phenotype and that 12/13 of these invasive tumours were all in TCB-treated animals. This demonstrates for the first time that one congener of PCB can enhance DMBA-induced mammary carcinogenesis and that PCBs can influence mammary as well as liver carcinogenesis [3, 4].

There is now a body of evidence implicating dietary fat in the development of DMBA-induced mammary cancer in rats [16–20] and the results of this experiment are in line with these previous findings. Several mechanisms have been put forward to explain the enhancement of rat mammary carcinogenesis by dietary fat including alterations to the immune system, prostaglandin synthesis, membrane fluidity, metabolic processes, cell sensitivity to hormones or growth factors [22] or simply higher calorific intake [16]. However, it is also conceivable that dietary fats could act by virtue of their ability to dissolve xenobiotic compounds such as PCBs. Dietary fat could act to increase levels of environmental pollutants in the body either by increased intake of chemicals dissolved in the dietary fat or by increased retention of compounds in the resulting elevated fat deposits of the body. The work here

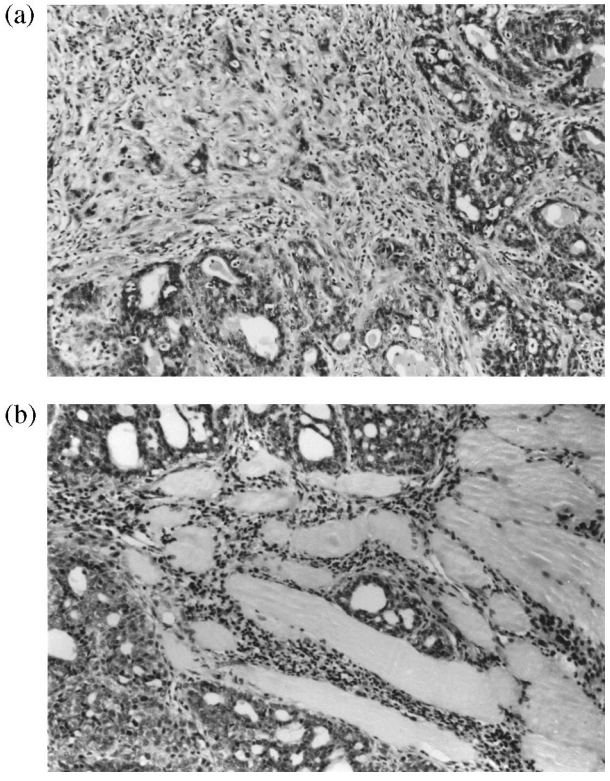

Figure 3. Histological appearance of two DMBA-induced mammary tumours from different rats, both treated with 3,3′,4,4′-tetrachlorobiphenyl (TCB) and fed on a high-fat diet. Stromal invasive ductal carcinoma of cribriform type showing (a) invasion in the left upper field and (b) invasion into skeletal muscle. Sections were stained with H&E, magnification ×150.

demonstrates that TCB could enhance mammary tumorigenesis in both low- and high-fat diets, but clearly tumour development was more aggressive in TCB-treated rats fed the high-fat diet in terms of both speed of development and invasive histological phenotype.

The lack of abnormalities in the liver in the face of aggressive mammary tumour development is unexpected in the light of published results of PCB-induced hepatocarcinomas [3, 4]. This could have been the result of different routes of administration (TCB was administered here by gavage rather than intraperitoneal injection) or due to the use of a single congener rather than a mixture of congeners. Alternatively, it may have been due to aggressive interaction of TCB with DMBA resulting in the development of mammary tumours long before the development of other abnormalities.

The mechanism of enhanced mammary tumour development by TCB in this study remains to be elucidated. One possible mechanism could result from the ability of PCBs to induce cytochrome P450 gene expression and in particular cytochrome P450 1A1, through an aryl hydrocarbon receptor (AhR)-mediated pathway [4, 23–25]. Since the carcinogenic action of DMBA is dependent on metabolic conversion by cytochrome P450 to the ultimate carcinogen DMBA-3,4-diol-1,2-epoxide which then reacts with DNA to form adducts [15, 26], the TCB could have acted by increasing cytochrome P450 1A1 gene expression [4] and thereby enhancing the rate of activation of DMBA to mutagenic epoxides. This would be consistent with previous studies showing that benz(*a*)anthracene, another AhR agonist and inducer of cytochrome P450 monooxygenases, can increase metabolic activation of DMBA in rat mammary epithelial cells [27]. However, this contrasts with studies using other AhR agonists 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and indole-3-carbinol where DMBA-induced mammary tumour growth was inhibited [28, 29]. In the case of TCDD, the mechanism of inhibition is suggested to relate to the anti-oestrogenic properties of this compound [28]. Despite the reported oestrogen antagonist activity of TCB under some circumstances [30], the overall physiological effect of TCB within this experimental protocol was to enhance the tumour growth and not to inhibit it. This would suggest that overall effects on DMBA-induced tumour growth result from interacting factors involving both differing properties of the chemicals and protocol of administration and which can give opposite end effects.

Although DMBA is a synthetic chemical not present either in the environment or in humans, there are other known environmental polycyclic aromatic hydrocarbons (PAHs) with a structural similarity to DMBA which may pose a human health hazard. Some PAHs, heterocyclic aromatic amines and nitro polycyclic aromatic hydrocarbons have been shown to induce mammary cancer in rats [16] and it will be important to determine the extent to which TCB could influence the action of these environmentally relevant chemicals in mammary carcinogenesis.

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