

# THE POSSIBLE EFFECT OF DIAZEPAM ON CANCER DEVELOPMENT AND GROWTH

Medical Hypotheses 7: 115-125, 1981

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## ABSTRACT

Diazepam has the characteristics of a tumour promoter in a number of *In Vitro* systems. The effect is apparent at concentrations of diazepam which are clinically relevant. Diazepam also accelerated tumour growth in two different experimental animal cancers. Tranquilizer use was found to be greater in women with metastatic breast cancer at the time of diagnosis than in those without metastases. Further evaluation of the possible effects of diazepam and related drugs on human and animal cancers is urgently required.

## INTRODUCTION

Two broad classes of agents are believed to be involved in the development of cancer. Initiators or mutagens are believed to act by producing irreversible changes in the genetic material. Initiators themselves are able to cause cancer when present at an appropriate concentration. They are detected by conventional studies of carcinogenicity in animals or by *in vitro* methods such as the Ames test which monitors their ability to cause mutations in bacteria. Tumour promoters, in contrast, do not usually cause mutations and do not usually alone cause cancer. Promoters can, however, enhance the effects of initiators and they may enable cancer to develop as a result of a mutagen exposure which would normally not produce a tumour (1,2,3). Promoters may not be detected in conventional tests of carcinogenesis. They can be identified only if the promoter is administered in the presence of a sub-threshold dose of an initiator. Recently a good deal of effort has been expended in the attempt to devise *in vitro* tests which will be analogous to the Ames test in their ability to detect promoters rapidly (4-9).

The benzodiazepines are minor tranquilizers which are the most widely prescribed drugs in the world. They include diazepam (Valium), chlordiazepoxide (Librium) and a variety of similar but less well known compounds. In the United States the National Institute on Drug Abuse has estimated that 42% of the adult female population and 21% of the adult male population have used minor tranquilizers (10). A number of studies have shown that at any one time 15-30% of the adult female population are taking diazepam or a closely related drug. For example, a recent study of women in Etobicoke, a suburb of Toronto showed that 14.7 % of women had used some form of psychotropic drug in the previous 48 hours and 23.5% admitted to using such drugs relatively often (11). After diagnosis of breast cancer, minor tranquilizer use in women may rise 2-3 fold (12).

The evidence presented in this paper suggests that diazepam may be a tumour promoter at concentrations present in the blood of anyone who takes a standard dose of 5mg three times a day. This possibility requires urgent investigation since if correct, tranquilizer use may be a major contributor to the development of some types of cancer.

## INITIAL OBSERVATIONS

The superior mesenteric vascular bed of the rat is a useful preparation for investigating calcium-dependent processes (13-16). The state of contraction of vascular smooth muscle depends, other things being equal, on the availability of calcium ions to the contractile mechanism. Contraction can therefore be used as a form of "calcium electrode" of exquisite sensitivity. Certain agents such as noradrenaline and angiotensin cause contraction in the mesenteric bed, primarily by releasing calcium ions from an intracellular or membrane-bound source (13-16). Other agents, such as potassium ions, promote entry of extracellular calcium.

Ultra-violet radiation, a known carcinogen (17), which may have both initiating and promoting properties, has an unusual effect in this arterial preparation. It seems to reduce intracellular calcium release without affecting the entry of extracellular calcium (18). It also reduces the rate at which calcium appears to be removed from the cytoplasm to terminate a contraction. The net effect is that after irradiation, responses to noradrenaline are reduced in amplitude but prolonged whereas responses to potassium are unchanged in amplitude. This unusual pattern of events is also produced by 8-methoxypsoralen (18), an agent which sensitises to ultra-violet radiation and which has complex effects on cancer which include both initiation and promotion (19,20). Phorbol esters, the best known and most potent tumour promoters, had similar effects (21).

These results were disturbing because for other reasons we had been investigating diazepam and chlordiazepoxide and had found them to behave in a way similar to that of the UV radiation and the phorbol esters (22). There is a good deal of evidence that calcium disturbances are present in cancer cells and that they may play a key role in the development of the malignant state (23-26). The effects of ultra-violet radiation, of 8-methoxypsoralen and of phorbols on calcium-dependent processes in muscle might therefore be indicators of actions which could be relevant to the development of cancer. If so, then diazepam might also be a tumour-promoting agent. We decided to test the possibility that it could modulate the growth of tumours.

## ANIMAL EXPERIMENTS

Diazepam and chlordiazepoxide were both known to be able to stimulate the abnormal development of mammary tissue in rats (27, 28). An animal model of breast cancer therefore seemed a potentially useful test system. We investigated the effect of diazepam on the growth of the R3230AC mammary tumour in rats. Equal-sized pieces of this transplantable tumour were placed subcutaneously and the animals treated daily with control injections or with approximately 2.5mg/kg/day of diazepam (21). After four weeks the tumours in the control group weighed 1.39g +/- 0.20 (SEM) and those in the diazepam treated group weighed 3.47g +/- 0.30. The two groups were very highly significantly different at the  $p < 0.001$  level.

Karmali, who played a major role in these initial experiments, repeated the studies after moving to another institution. Diazepam at 2.5mg/kg/day, significantly increased the size of the R3230AC tumours, but at 5mg/kg/day, there was no significant effect (29). In the Walker 256 tumour transplanted under the kidney capsule in rats, 1.7mg/kg/day of diazepam significantly increased tumour size, whereas 4.2mg/kg/day had no significant effect (30).

There was thus the unusual phenomenon of an agent enhancing tumour growth at a low dose but not at a higher one in two different animal cancers. When this happens, cancer experts often state that there is no dose response effect and therefore that the results are either chance events with no biological meaning or are the product of toxicity at higher doses. For example, a reviewer of one of our submissions to the Lancet on this topic recommended rejection on the grounds that "there was no dose/response effect". This is strange reasoning, especially since it is well known that the radiation and many of the chemicals used to destroy cancer therapeutically are themselves carcinogenic when given at lower doses. It is a truism in the pharmaceutical industry that if one wants to find an anti-cancer drug, a good place to start is with agents that cause cancer.

Bell-shaped dose response curves, meaning effects which are not present at either low or high concentrations, but which are detected at intermediate levels, are actually common features of drug and hormone action. Their physiological basis has been frequently discussed (31-34). Kon has made a particular study of this in the cancer field and has shown that many agents can cause cancer at low doses but not at high ones (34). This phenomenon seems to be characteristic of some promoter systems and it is not uncommon for promoters to produce fewer tumours at high doses than at lower ones (2,3). Phorbol esters are also more effective at inducing persistent infection with oncogenic viruses at intermediate concentrations than at higher or lower ones (56).

The fact that an effect of diazepam on animal tumour growth disappears at higher doses is therefore not an exoneration of the benzodiazepines. The only published long term animal study of diazepam that we have been able to find used 10mg/kg as the lowest dose (35), more than double the higher dose used in Karmali's experiments. However it should be noted that another benzodiazepine, oxazepam, has been reported to have no effect on experimental mammary cancer (36). In unpublished work we have been unable to find any significant enhancement of growth of the R3230AC tumour in response to the tricyclic antidepressant, clomipramine.

## **HUMAN BREAST CANCER**

There are rare reports of benzodiazepines causing abnormal breast growth and lactation in both male and female humans (37-39). Such an effect is consistent with the possibility that these agents may accelerate breast cancer growth. Stoll is interested in the effects psychological factors on cancer growth (40,41). He identified a group of women who had taken tranquilizers prior to the development of breast cancer. He also noted that tranquilizer use rose sharply after diagnosis. The women who had used tranquilizers prior to diagnosis were significantly more likely to have axillary metastases at the time of diagnosis and significantly more likely to have a recurrence within a year. Stoll interpreted this as indicating that mental status may influence the development and/or growth of breast cancer. One of several alternative explanations is that breast cancer growth is modified by tranquillizer use (58). Greer et al investigated the effect of mental attitude after diagnosis on the outcome in breast cancer (42). They divided the patients into four groups, those who denied they had cancer, those who had a fighting spirit, those who were distressed and then stoical and those who had feelings of hopelessness. The first two groups did significantly better and it was felt that a likely explanation was the effect of mental state on cancer growth. Drug use was not recorded but it seems highly probable that tranquillizer use was substantially higher in the two groups who did poorly. The American Cancer Society 20 year study also indicated that cancer seemed more common in those who used tranquillizers while another study could find no association (68,69).

## TERATOGENIC EFFECTS OF BENZODIAZEPINES

There is some evidence that agents which enhance cancer development can also act as teratogens either like initiators by modifying the genetic material (60, 61,62) or like promoters by interfering with cell surfaces and cell-cell communication (63,64,65). Breen et al (43) found that high concentrations of diazepam could produce chromosome breaks in human fibroblasts. Bandman et al (44) found that diazepam could block a normal stage in the development of fetal muscle. In animals there is evidence that psychotropic drugs including diazepam may act as behavioural teratogens (45,66). If diazepam can block cell- cell communication (see below) then in view of the importance of such communication in nervous system development, an effect on the fine structure of the nervous system and on later behaviour is not surprising.

There have been several surveys of the possible teratogenic effects of benzodiazepines in humans. The Boston Collaborative Drug Surveillance Program (46) using recall by mothers themselves as an investigative method, could find no evidence of a relationship between maternal use of minor tranquillizers and fetal abnormalities. But another study, based on actual inspection of patient drug records, suggested that benzodiazepines might indeed be associated with teratogenesis (47). Two further reports supported this view, suggesting that some fetal abnormalities, notably cleft lip and palate, were substantially more common in children of mothers who had taken benzodiazepines early in pregnancy (48,49).

## DIAZEPAM IN TUMOUR PROMOTER TESTING SYSTEMS

The most rigorous way to test a scientific hypothesis is to make a proposal which is highly improbable yet which can be easily experimentally tested. A number of investigators have devised ways of testing for promoting activity and in the winter of 1979-80 several were informed of the hypothesis that diazepam might be a tumour promoter and asked to test it out. Three results are available to date and all are positive.

1. Cooperation between two lines of Chinese hamster lung cells (6-9). It has been proposed that tumour promoters may act to block metabolic cooperation between cells. There is evidence that normal cells may be able to produce substances which when transferred to potentially malignant cells can hold proliferation in check and hence prevent the development of a tumour. The assay is based on this concept and shows excellent correlation with other measures of promoting activity based on conventional in vivo studies (6-9).

**Table 1.** Recovery of 6TG-resistant cells in the presence of 12-O-tetra- decanoyl-phorbol-13-acetate (TPA) and varying concentrations of diazepam indicates that treatment significantly increased the percentage recovery above control at p.(0.01 or better according to the Student-Newman-Keuls test. Results are expressed as means +/- SEM.

<u>6TG sensitive cells-no</u>	<u>6TG resistant cells-no</u>	<u>Drug</u>	<u>Drug conc. Ng/ml</u>	<u>% recovery of resistant cells</u>
9x 10 <sup>5</sup>	100	Control	0	13.1 ± 1.35
9x10 <sup>5</sup>	100	TPA	100	101.5 ± 4.14 *
9x10 <sup>5</sup>	100	Diazepam	500	20.2 ± 1.31 *
9x10 <sup>5</sup>	100	Diazepam	750	21.8 ± 1.38 *
9x10 <sup>5</sup>	100	Diazepam	1000	23.6 ± 1.43 *
9x10 <sup>5</sup>	100	Diazepam	1250	31.2 ± 2.28 *

The assay involves the co-cultivation of 6-thioguanine (6TG)-sensitive and 6TG-resistant Chinese hamster V79 lung cells in the presence of 6TG. 6TG added to cells which contain the enzyme hypoxanthine guanine phospho-ribosyl-transferase (HGPRT) phosphorylate and incorporate the 6TG and are killed. Resistant cells which lack the HGPRT and so cannot phosphorylate 6TG are not killed by 6TG. When the two cell lines are grown together, the phosphorylated analogue can be transferred from the sensitive to the resistant cells by way of gap junctions. The resistant cells thus become sensitive to 6TG killing. Tumour promoters block this cooperation and allow the 6TG-resistant cells to escape, even when they are cultured with the sensitive strain. The percentage of 6TG-resistant cells recovered after co-culture is used as a measure of tumour-promoting activity which shows good correlation with conventional studies. The results (50), re-produced in table 1, demonstrate that diazepam has a highly significant effect in blocking cell cooperation. The effect is weak compared with that of TPA, one of the most potent tumour promoters known but it is about 10,000 times stronger than that of saccharin, which has recently received a good deal of attention as a cancer-causing agent (9). Even more important, the threshold of the effect was at a concentration (500ng/ml), which is readily achieved in human plasma by ordinary doses of diazepam. 500ng/ml is a concentration reached following a single oral dose of 20mg (51), while 20mg intravenously produced a peak concentration of 1600ng/ml. A dose of 5mg three times a day, common in diazepam therapy and commonly exceeded, resulted in a mean diazepam serum concentration of 250ng/ml plus a concentration of 350 mg/ml for the pharmacologically active metabolite, N-desmethyldiazepam (52).

**2. Blockade of terminal differentiation.** There is good evidence that an action which tumour promoters have in common is blockade of terminal differentiation in a variety in cell lines (4,5). Using a melanoma cell line, I.B. Wienstein and colleagues have found that diazepam, like known tumour promoters, is effective in blocking such differentiation.

**3. Ability to interact with tumour promoter receptors.** Smythies has constructed models of the chemical structure of a site on phospholipase A2 which seems to be able to bind phorbol esters with high affinity (54). The chemical structure of diazepam is such that it could bind to this receptor.

## **OTHER POTENTIALLY RELEVANT EFFECTS**

Clarke et al (57) recently found that diazepam and other benzodiazepines could induce differentiation in Friend cells and could block mitogenesis in 3T3 cells concentrations of 35-40 ug/ml. They interpreted this as indicating that diazepam might have anti-cancer activity. However these concentrations are far higher than those used in the Chinese hamster ce11 system and bearing in mind biphasic in vivo effects it is possible that lower concentrations might have opposite action. Opposing effects seem to be characteristic of tumour promoters which may induce differentiation in one cell line and block it in another (67). Induction of differentiation is therefore not a certain guide to a tumour inhibiting activity. Moreover, radiation and many drugs have anti-cancer effects at high concentrations, but are carcinogenic at low ones. Benzodiazepines have also been found to have radioprotective effects (59). This observation appears not to have been followed up possibly because a number of known radioprotective agents are rather toxic.

## **COULD BENZODIAZEPINES AFFECT CANCER DEVELOPMENT AND GROWTH IN HUMANS?**

At present the most direct evidence in humans is the work of Stoll(40,41). The American Cancer Society (69) and the Friedman et al (68) studies give contradictory information. The ubiquity of minor tranquilizer use and the fact that people do not regard these agents as drugs will make epidemiological surveys difficult and all but the most careful work useless. But the potential importance of the problem makes it imperative that such surveys be done. Of particular importance is the fact that tranquilizer use rises sharply after cancer diagnosis (40).

Many patients are therefore being treated simultaneously with radiation and drugs which are known cancer initiators and with tranquilizers, which seem to be promoters. If tranquilizers are promoters, one devastating consequence would be that almost all cancer studies over the last 20 years may be useless. Substantial numbers of patients in such studies are on these drugs, but no attempt is ever made to match groups for tranquilizer use. The studies may therefore be invalidated.

Malignant melanoma may be a cancer particularly worth investigating. It is easily identified and is probably mainly caused by ultraviolet radiation (17). 8-methoxypsoralen, an agent with an action on muscle similar to that of diazepam is known to be both an initiator and promoter and to enhance the effects of ultraviolet radiation (18-20). Weinstein's work on diazepam was done in a melanoma cell line. The incidence of melanoma has recently risen sharply in most light-skinned Western populations. A recent study documented these changes in detail for England and Wales during the years 1952-75 (17). Two trends were apparent. First, melanoma incidence was positively correlated with the mean daily hours of sunshine recorded two years earlier, suggesting that ultra-violet radiation is involved in the causation of melanoma. Secondly, in both sexes but especially in women, the total melanoma incidence has risen substantially. Regression lines were drawn through the data from the Oxford region right from 1952 through to 1975. Reanalysis of the data shows that the trends can be broken down into two distinct periods, before and after 1962. Regression lines for the earlier period alone show no trend in males and only a slight rise in females. Regression lines for the later period show a very substantial rise in incidence in both sexes. Both earlier and later periods show the correlation with sunshine two years previously.

It is possible but unlikely that the amount of ultra-violet and other radiation reaching the earth or generated on the earth changed suddenly in the late 1950s or early 1960s. A more likely concept is that around that time something happened which promoted the carcinogenic effects of ultra-violet radiation. There are many possibilities including increased sunbathing and increases or changes in the types of agents used to influence sun tanning. This was also precisely the time at which benzodiazepines began to be used on a large scale. Studies of benzodiazepine use in patients with melanoma may therefore be of value.

## **CONCLUSIONS**

Evidence from many different sources now suggests that diazepam and possibly other benzodiazepines have actions similar to those of known cancer promoters. These actions can occur at concentrations which are directly relevant to the clinical situation. Further in vitro and animal studies aimed at determining the chemical structure responsible for these effects and epidemiological studies in humans are urgently required. In the meantime it would seem prudent to warn

populations who already have cancer, or those who are at particular risk of developing cancer, of the possible implications of tranquilizer use.