*Eur. J. Cancer Clin Omal* Vol. 17, No. 7, pp. 767–773, 1981.
Printed in Great Britain.

0014-2964/81/070767-07 $02.00/0


# Hormonal Induction of Kidney Tumours in Male Hamsters. Inhibition of Growth by a Phenothiazine Derivative (Perphenazine)*

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**Abstract**—*Experiments have shown that long-term treatment of male hamsters with oestrogen will cause renal carcinomas, neoplastic changes in the pars intermedia of the pituitary and highly elevated levels of melanocyte-stimulating hormone (MSH). To test if MSH was fundamentally involved in renal carcinogenesis, a group of hamsters was treated for* 317 days *with perphenazine which is known to elevate MSH levels. A second group was treated with oestrogen while a third received a combination of oestrogen and perphenazine for a similar period of time. Serum MSH levels were significantly elevated in the perphenazine group but kidney tumours were absent. All oestrogen-treated animals developed kidney tumours but only* 19% *of those given the combined treatment developed kidney tumours despite highly elevated levels of MSH. The findings suggest that MSH does not play a significant role in renal carcinogenesis in the hamster. Perphenazine may inhibit the induction of oestrogen-induced tumours by competing with oestrogen for receptors in the kidney or may protect cells from toxic injury by stabilising cell and microsomal membranes. The phenothiazines are known to induce, and increase the activity of, microsomal enzymes and that may lead to enhanced detoxification of the carcinogen. Other effects of phenothiazines include elevation of prolactin levels. The fact that the body weight of animals treated with DES and perphenazine was significantly lower than that of the other groups may indicate that the reduction in tumour incidence was the result of a certain degree of undernutrition. Further work is required to elucidate the mechanism of tumour inhibition by phenothiazine derivatives.*

## INTRODUCTION

RENAL carcinomas may be induced in male hamsters by long-term treatment with oestrogens [1–3]. The mechanism of carcinogenesis is unclear. Induction may result from the direct effect of oestrogen on the kidney or may be mediated via the pituitary, since marked changes in that organ have been reported following oestrogen treatment of hamsters [4, 5]. In particular, such treatment results in increased production of melanocyte-stimulating hormone (MSH) [3, 6]. Since MSH is involved in maintenance of electrolyte balance [7] and appears to be metabolized exclusively by the kidney [8, 9], it was considered important to investigate the effects of chronic elevation of plasma MSH levels with regard to renal carcinogenesis. Phenothiazine derivatives are known to elevate MSH levels in experimental animals [10] and one such compound, perphenazine, was chosen for this purpose.

## MATERIALS AND METHODS

Four groups of male Syrian golden hamsters (*Mesocricetus auratus*), aged 3–4 months at the beginning of the experiment, were used. Group I (eight animals) were injected subcutaneously with 0.5 mg perphenazine (Fentazin, Allen & Hanburys Ltd.) in 0.1 ml sterile distilled water five times per week over a period of 317 days. Group II (eight animals) were injected subcutaneously with 0.6 mg diethylstilboestrol (DES, Sigma) in 0.2 ml sterile distilled water three times per week. Group III (eight animals) were given both perphenazine and DES in the same

Accepted 5 February 1981.
*This work was made possible by the financial support of the Yorkshire Cancer Research Campaign.

manner as Groups I and II, respectively. Group IV (fourteen animals) was a control group and these animals were given 0.2 ml sterile distilled water five times per week by subcutaneous injection. The animals were housed individually in a light- and temperature-controlled room and maintained on Purina dog chow and tap water *ad libitum* supplemented twice weekly with cabbage and flower seeds.

After receiving a total dose of 81 mg DES and 112.5 mg perphenazine over a period of 317 days, the animals were weighed, killed by cervical dislocation and autopsied. Blood samples were collected and sera were separated and stored at −20°C for future estimation of MSH concentration. Kidneys and testes were weighed and fixed in 10% formol saline along with specimens of pancreas, liver, prostate, seminal vesicle, vas deferens, thyroid, adrenal and skin. Pituitary glands were weighed and placed in separate phials containing 1 ml of 0.25% glacial acetic acid and stored at −20°C for future estimation of MSH content. The method of Howe and Thody [11] was used for the bioassay of total MSH activity in extracts of pituitary tissue. A radioimmunoassay was used for the measurement of pituitary and serum MSH [12, 13]. Statistical analysis was done using a Data General Corporation Nova 820 computer programmed to correct for inter-group variance differences when computing $P$ values.

In addition to the above four main groups of animals, a number of extra male hamsters were used as follows: (a) eight animals were given DES + perphenazine in the same manner as Group III. These animals were used for electron microscopic studies of kidney and testis and for cytological studies of the pituitary; (b) eight animals were given DES as in Group II and were used for pituitary cytology; (c) five animals were given perphenazine as in Group I and were used for pituitary cytology; (d) three control animals injected with sterile distilled water were used for pituitary cytology. Kidney tissue was prepared for electron microscopy by fixation in 2% glutaraldehyde in Millonig's phosphate buffer, post-fixation in 1% osmium tetroxide and embedding in Epon 812. Pituitaries were fixed in formol sublimate and stained by the tetrachrome method of Herlant [14] and by periodic acid–Schiff [PAS] and hematoxylin. Complete data were not available from these extra animals but the kidneys were submitted to gross and histopathological examination for the presence of tumours.

## RESULTS

Table 1 records the body and organ weights of animals of Groups I–IV. The mean body weight of animals of Group III (DES and perphenazine) was significantly less than that of other groups. This difference was attributable to a paucity of depot fat in the mesenteries and epididymal fat pads. There were no significant differences in mean body weight among the other three groups of hamsters.

*Kidneys*

From Table 1 it may be seen that the kidneys from animals of Groups II (DES) and III (DES and perphenazine) were significantly heavier than those of animals in the control and perphenazine-treated groups. Although statistical analysis failed to show a significant difference between mean kidney weights of the DES and the DES and perphenazine groups, the mean kidney weight in the latter group was less than half that of the DES-treated group and the lack of statistical significance is due to small sample numbers combined with disproportionally large S.E.M. in the DES-treated group.

There were no kidney tumours in control or perphenazine-treated hamsters. Of the 16 animals given DES, all had renal tumours (Table 2), a fact that accounted for the increased kidney weight in Group II. Histopathological examination showed the tumours to be invasive carcinomas originating from proximal convoluted tubules of the kidney. There was little evidence of interstitial reaction or infiltration by macrophagic, lymphocytic or other cell types. Metastases occurred in two of the DES-treated animals and affected the serosal surfaces of abdominal organs and the peritoneal surface of the diaphragm and were associated with haemorrhagic ascites.

Of the 16 animals given DES and perphenazine, only three showed any gross or histopathological evidence of kidney tumours. Of these, two animals had a unilateral microfocus while the other showed two small nodules of 2 and 3 mm diameter in each kidney.

*Pituitaries*

Table 1 shows the pituitary weights in Groups I–IV. There was no significant difference in pituitary weight between the control and perphenazine-treated groups. In animals given DES, the pituitaries were significantly heavier than those of the controls or perphenazine-treated animals ($P<0.001$). The

*Table 1. Effect of diethylstilboestrol (DES) and perphenazine (Pe) on weight* of body and organs in male hamsters*

| Group | No. of animals | Body (g) | Kidneys (g) | Pituitary (mg) | Testes (g) |
|---|---|---|---|---|---|
| I | 8 | 121±5 | 0.5911 ± 0.0188 | 2.6±0.3 | 1.8272 ± 0.1275 |
| II DES | 8 | 120±5 | 1.6900 ± 0.5230 | 41.1±1.8 | 0.0853 ± 0.0084 |
| III DES+Pe | 8 | 106±2 | 0.8189 ± 0.0425 | 24.1±6.5 | 0.0879 ± 0.0069 |
| IV Control | 14 | 130±4 | 0.5668 ± 0.0163 | 3.0±0.3 | 1.9466 ± 0.0804 |

*Expressed as mean ± S.E.M.

*Table 2. Effect of diethylstilboestrol (DES) and perphenazine (Pe) on renal carcinogenesis in male hamsters*

| Treatment | No of animals | No. of animals developing kidney tumours | Maximum tumour diameter | No. of animals with metastases |
|---|---|---|---|---|
| Pe | 13 | 0 | — | — |
| DES | 16 | 16 | 4 cm | 2 |
| DES+Pe | 16 | 3 | 3 mm | 0 |
| Controls | 17 | 0 | — | — |

pituitaries of hamsters given DES and perphenazine were heavier than those of the controls or of the perphenazine-treated group ($P<0.001$ and $P<0.005$, respectively), although this increase in weight was significantly less ($P<0.02$) than that occurring in the group given DES alone. In that group, the increased size and weight of the pituitary were related to hypertrophic, hyperplastic and neoplastic changes in the intermediate lobe. In animals treated with perphenazine alone, although there was no increase in the weight or size of the intermediate lobe, there was a marked increase in the relative proportions of "light" cells which were hypertrophied with larger, more vesiculate nuclei and more conspicuous nucleoli.

Examination of the anterior lobe of pituitaries of DES-treated animals showed a great increase in the number of prolactin cells and a decrease in the proportion of growth hormone cells and basophils, the chromophobes remaining unchanged. Treatment with perphenazine alone did not alter the number of prolactin cells or growth hormone cells but the latter, instead of staining a normal bright yellow-to-orange colour with Herlant's stain, displayed a muddy orange colour and, under oil immersion, were seen to be staining amphoterically. There was a moderate increase in the proportion of light-staining basophils (presumptive gonadotrophs) with, unusually, frequent mitosis, while the dark-staining basophils (presumptive thyrotrophs) appeared hypertrophied with enlarged nuclei. The anterior lobes of animals treated with DES and perphenazine showed a striking increase in the number of growth hormone cells that stained a bright orange colour and conferred a distinctly acidophilic appearance to the lobe, in contrast to the basophilic aspect seen in control glands.

*Testis*

Animals treated with DES showed severe testicular atrophy and significant reduction in the weight of this organ (Table 1). The addition of perphenazine (Group III) did not have any sparing effect on testicular weight or atrophy. The administration of perphenazine

alone produced no significant change in testicular weight and no alteration in the histological appearance of the testis.

*Melanocyte-stimulating hormone*

Table 3 and Fig. 1 show the pituitary and serum levels of MSH. Perphenazine increased serum concentration of the hormone but decreased pituitary content. DES and a combination of DES and perphenazine increased both serum and pituitary levels of MSH. There were no significant differences in serum or pituitary levels between the DES-treated animals and those given DES and perphenazine.

*Table 3. Effect of diethylstilboestrol (DES) and perphenazine (Pe) on α-MSH levels* in male hamsters*

| Group | No. of animals | Pituitary MSH (ng/gland) | Serum MSH (pg/ml) |
|---|---|---|---|
| I Pe | 8 | 267±52 | 225±16 |
| II DES | 8 | 5279±519 | 986±119 |
| III DES+Pe | 8 | 4148±421 | 907±103 |
| IV Controls | 14 | 436±41 | 179±10 |

*Expressed as mean±S.E.M.

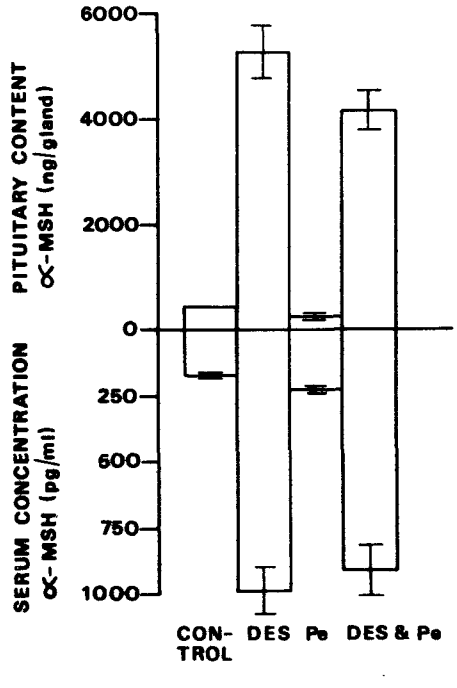


*Fig. 1. Effect of DES and perphenazine on pituitary and serum levels of α-MSH in male hamsters. The vertical lines indicate the standard error of the mean.*

## DISCUSSION

Perphenazine, a phenothiazine derivative that is used widely as a pshychotherapeutic agent, inhibited the incidence and severity of oestrogen-induced kidney tumours in male hamsters. The mechanism by which this was achieved is speculative. It is known that perphenazine competes with oestrogen for receptors in the pituitary and the hypothalamus [15], although there is no information concerning receptor competition in the kidney, where oestrogen receptors are also present [16]. It is worth noting that testicular atrophy and pituitary enlargement still took place in animals given a combination of DES and perphenazine so that competition for oestrogen receptors did not lessen the changes characteristic of oestrogen treatment in the hamster. Further work is required to elucidate the importance of oestrogen receptor saturation in the inhibition of renal carcinogenesis by perphenazine.

Phenothiazine derivatives prevent disturbance of phospholipid metabolism with its associated membrane dysfunction and thereby stabilize cell and microsomal membranes and obstruct the inflow of excessive amounts of calcium into cells damaged by a variety of noxious agents [17, 18]. Phenothiazines also induce microsomal enzymes and Wattenberg and Leong [19, 20] reported that, by increasing the amount of polycyclic hydrocarbon hydroxylases, these drugs protected animals against DMBA carcinogenesis. Pamukcu *et al.* [21] showed that perphenazine increased the production of hepatic and intestinal mucosal benzpyrene hydroxylases with a reduction in the incidence of bladder and intestinal tumours in bracken-fed rats. The authors suggested that the hydroxylases may have caused enhanced detoxification of the carcinogen but did not dismiss the possibility of direct interaction between perphenazine and the carcinogen or, possibly, competition for binding sites. Julou *et al.* [22] were unable to demonstrate an anti-tumour function of chlorpromazine and other phenothiazine derivatives in transplanted tumours in mice, with the exception of the Ehrlich tumour in which inhibition of growth was ascribed to an anti-exudative effect of the drug. On the other hand, Force and Liu [23] recorded significant inhibition of growth of transplanted tumours and regression of solid tumours in mice treated with chlorpromazine, and Kruger *et al.* [24] reported increased survival in mice with L1210 leukemia that had been similarly treated. In contradistinction, Pearson *et al.* [25]

demonstrated that perphenazine enhanced the induction of DMBA mammary tumours in rats. Such conflicting reports of anti-tumour activity may be explicable on the grounds that two major functions of perphenazine—the mammotrophic and the sedative effects—are entities that can be blocked individually [26]. Hence the prolactin-stimulating effect that is important in DMBA mammary carcinogenesis [27] may overwhelm other potentially anti-tumour effects that perphenazine may possess for that particular tumour system. Whether or not one of the above mechanisms were responsible for the tumour inhibition in the present experiment is a subject for further investigation.

Melanocyte-stimulating hormone is markedly increased in the pituitary and serum of oestrogen-treated hamsters [5] and this observation gave rise to the suggestion that MSH may have been involved in the genesis of the oestrogen-induced kidney tumours. However, the animals of the present experiment given DES and perphenazine showed similarly increased pituitary and serum levels of MSH but, despite this, carcinogenesis was greatly suppressed. Animals treated only with perphenazine developed elevated serum MSH but no kidney tumours. These findings indicate that MSH plays no significant part in the induction of kidney tumours.

It is well established that perphenazine increases serum prolactin levels in man and laboratory animals, acting by blocking dopamine receptors and so preventing dopamine-mediated inhibition of prolactin synthesis [28]. This suggests the possibility that hyperprolactinaemia may be inhibitory to renal tumour induction. However, in a previous experiment [4] it was found that bromocryptine, an inhibitor of prolactin synthesis and secretion, suppressed DES-induced renal carcinogenesis in hamsters.

Prolonged oestrogen administration results in marked atrophy of the testes. However, in animals treated with perphenazine in combination with DES, despite the reduction in tumour incidence, the testicles were still atrophic. These findings indicate that androgen deficiency is not involved in the induction of this tumour.

In a previous experiment, Hamilton *et al.* [4] found that bromocryptine, which suppressed DES-induced renal carcinogenesis, also produced increased numbers of growth hormone cells when compared with DES-treated animals. In the present experiment, perphenazine had a similar effect to that of bromocryptine with regard to tumour suppression and there was also an associated increase in the number of growth hormone cells in the anterior lobe. However, it is possible that those cells may have been synthesizing, but not secreting, growth hormone since, according to Sherman *et al.* [29], the phenothiazines as a group inhibit growth hormone releasing factor. Nevertheless, the above results indicate that the role of growth hormone in the genesis of oestrogen-induced renal tumours requires investigation.

The body weight of animals treated with DES and perphenazine was significantly less than that of other groups (Table 1) but as food intake was not monitored it is impossible to say whether this resulted from depressed appetite induced by the drug combination or from increased body metabolism. It is generally recognised that, in experimental systems, overnutrition favours, and undernutrition inhibits, tumour incidence [30, 31]. The effect of diet on the experimental induction of kidney tumours was illustrated by Hamilton and Saluja [32] who reported increased incidence of renal tumours in hamsters fed an enriched diet. It is, therefore, possible that the reduction in tumour incidence associated with the addition of perphenazine to DES in the present experiment may have arisen from a certain degree of undernutrition in the animals and this factor is worthy of further examination.

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