

INTERSPECIES RESPONSE TO CARCINOGENS AND OESTROGENS

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Summary—Carcinogenic chemicals produce specific malignancies in a wide variety of animal species and in a high proportion of the treated animals. Oestrogens do not demonstrate a carcinogenic profile, producing malignancies in a limited number of animal species and in a low proportion of the treated animals. The effect of oestrogens depends on both species and strain.

Introduction

Dr Leon Golberg has contributed to toxicology not only as the founder and longtime Editor of *Food and Cosmetics Toxicology*, but also by his research and publications (cf. Golberg, 1974, 1978).

It may be remembered that Dr Golberg was one of the discoverers of diethylstilboestrol, the first synthetic oestrogen to be developed (Dodds, Golberg, Lawson & Robinson, 1938). The oestrogens are often cited as being carcinogenic in animal studies, and in this brief review I should like to summarize responses of a range of species to oestrogens and to contrast the results with the effects of carcinogens. The discussion of the effects of oestrogens will be limited to their action on the breast and the uterine cervix and endometrium.

Interspecies response to carcinogens

Compounds such as 2-naphthylamine, aflatoxin B₁, asbestos, and the other examples listed in Table 1, produce their characteristic tumorigenic effect in a number of different species (IARC, 1972, 1974, 1976 & 1977; Irving, Wiseman & Young, 1967; Miller & Enomoto, 1964; Miller, Miller & Hartman, 1961). Clearly the response to a specific carcinogen is not limited to one or two species but, allowing for differences in

absorption and metabolism, is a general biological response obtained in a variety of species. Indeed, based on the concept of covalent binding to DNA, we would expect a carcinogen to produce essentially the same effect in different species of animals. When a given compound induces the same carcinogenic response in several different species it may be predicted, depending on dosage and duration of exposure, that a carcinogenic response will also be obtained in man.

Two other characteristics of carcinogens should also be noted:

- (i) Carcinogens induce their specific neoplastic effects in a high proportion of the treated animals (Table 2).
- (ii) The increased incidence of specific neoplasms is obtained in a relatively short period of time. The neoplastic response is discerned before the end of an 18-month toxicity study in the mouse or a 2-yr chronic toxicity study in the rat.

Interspecies response to oestrogens

The effect of oestrogens on the occurrence of malignancies of the mammary gland, uterine cervix and endometrium in different species of animals is summarized in Table 3. It is apparent from this summary

Table 1. Examples of species' responses to carcinogens

Compound*	Target site	Carcinogenic response of							
		Mouse	Rat	Hamster	Guinea-pig	Rabbit	Dog	Monkey	Man
2-Naphthylamine	Bladder	+	0	++			+	+	+
4-Aminobiphenyl	Bladder	+	+			+	+		
MNNG	GI tract	+	+	+		?	+		
N-OH-AAF	GI tract	+	+	+	+	0			
Aflatoxin B ₁	Liver	++	+					+	+
Asbestos†	Pleural mesothelium, lung	+	+	+		+			

+ = Positive effect 0 = No effect

MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine N-OH-AAF = N-Hydroxy-2-acetylaminofluorene

*Administered orally unless otherwise stated.

†Intraperitoneal, ineffective orally in the mouse.

‡Inhalation.

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Table 2. Time and intensity of carcinogenic response. Effect of N-hydroxy-2-acetylaminofluorene in mice and rats

Target site		Percentage of animals with cancer			
		In female mice* after ...	10 months	12 months	14 months
		treated	9	19	30†
Mammary gland.		control	7.5	12.5	15†
Forestomach‡			2	12	23
Liver‡			5	5	12
Urinary bladder‡			0	2	9
		In rats* after ...	3 months	5 months	8 months
Mammary gland‡	M				0
	F				67
Forestomach‡	M				9
	F				60
Liver‡	M		27	45	64
	F		0	0	13

*Mouse data from Miller *et al.* 1964; rat data from Miller *et al.* 1961.

†Survival rates of the treated and control groups differed; life-table analysis is necessary.

‡Data is for treated animals only since there were no malignant tumours in control mice at 14 months, or in control rats at 8 months.

that oestrogens do not produce comparable effects in several different species or a response of the magnitude that is characteristic of a carcinogen. A positive effect of oestrogen is restricted to certain special situations.

Breast cancer

Articles on hormones and cancer may state that exogenous oestrogen causes breast cancer in mice, but such a statement is only partly true. It is usually not recognized or appreciated that oestrogen will increase the incidence of breast cancer only in *male* mice from inbred strains that possess a transmissible milk factor (a mammary tumour virus); oestrogen is ineffective in male mice from a low-tumour strain, i.e. without the milk factor. Neither mestranol nor ethinyloestradiol increases the occurrence of mammary cancer in CF-LP females, a low-tumour strain (Committee on Safety of Medicines, 1972; Drill, 1974; Lee, 1979).

The effect of oestrogen in the female rat is very variable. As indicated in Table 3 oestrogen may be without effect or may produce mammary cancer in as many as 27% of the treated animals. The reason for this marked variation is unknown although it may possibly represent in part the variation that is encountered in untreated female rats. For example, Durbin *et al.* (1966) found the normal incidence of mammary adenocarcinoma in untreated rats to vary between 0 and 18%, and in our studies the probability of tumour development based on life-table analysis varied from 0 to 15.7% (Drill, 1974).

References to studies of breast cancer in other species are cited in several reviews (Drill, 1975 & 1981). Of particular interest is the absence of an effect of oestrogen in dogs and monkeys treated with different doses of oestrogen for 6 or more years.

A variety of clinical studies also demonstrates the lack of a relationship between exogenous oestrogen and breast cancer. In five retrospective case-controls there was no association between use of an oestrogen and the relative risk for breast cancer, and a series of 12 clinical studies did not demonstrate an increased occurrence of cases of breast cancer in women treated with oestrogens (cf. Drill, 1975 & 1981).

Cervical cancer

The oral administration of oestrogen may occasionally induce cervical carcinomas in mice, but oestrogens do not produce cervical cancer in other species (Drill, 1976). Even in the mouse the overall incidence of cervical carcinomas is low and is not characteristic of the effects of a carcinogen (Table 3). Clinical studies do not provide any evidence that the long-term use of oestrogen induces cervical cancer (Drill, 1976), indicating good agreement between the experimental and clinical data.

Endometrial cancer

The relationship of exogenous oestrogen to endometrial cancer is currently the subject of intensive clinical study. However, oestrogens do not act as typical carcinogens in animals, producing a slight

Table 3. Comparison of carcinogenic effects of oestrogens in female animals

Target site	Carcinogenic response to oestrogens of ...						
	Mouse	Rat	Guinea-pig	Rabbit	Dog	Monkey	Man
Breast	0*	+†	0	0	0	0	0
Uterine cervix	± (1.4%)	0	0	0	0	0	0
Endometrium	± (2.1%)	0	0	+?	0	0	0?

0 = No effect ± = Borderline effect + = Positive effect

*Positive in male mice with mammary tumour virus.

†Range from 0% to 27% with adenocarcinoma.

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effect in the mouse and possibly a positive effect in the rabbit, but no effect in other species (Table 3). A strain difference in response may be present in mice, as the carcinoma was present in eight of 71 BDH-SPF mice and only in 3 of 461 other mice (Drill, 1979 & 1980). Carcinomas may possibly occur in the rabbit, but information in this species is very limited; the rabbit may be an atypical species as cancer may occur in as many as 56% of untreated rabbits in the Tans breed (Greene, 1959).

A complete review of clinical data on oestrogen administration and endometrial cancer is beyond the scope of this paper and only general comments will be made on the subject. Much of the clinical data is derived from retrospective case-control studies (which measure association), and in a tabulation of nine studies a risk was absent in four studies and present in five of the studies (Drill, 1980). In the study of Horwitz & Feinstein (1978) the relative risk varied from 1.2 to 1.7 depending on the sampling procedure (see also Horwitz & Feinstein, 1980).

More recently, an increase in risk has also been reported by Hulka, Kaufman, Fowler, Grimson & Greenberg (1980) and Shapiro, Kaufman, Slone, Rosenberg, Miettinen *et al.* (1980) and some authors have found a higher risk with increasing duration of use of oestrogens. In contrast, a series of retrospective case-control studies conducted in Europe has failed to demonstrate a risk for endometrial cancer in oestrogen-users (Lauritzen, 1978; Salmi, 1979 & 1980; Völker, Kannengiesser, Majewski & Vasterling, 1978). Antunes, Stolley, Rosenheim, Davies, Tonascia *et al.* (1979) found a relative risk of either 6.0 or 2.1 depending on the control group selected, indicating again the variation in results that can be obtained. Ultimately, prospective clinical data will be required if we are to reach a definite conclusion on oestrogens and endometrial cancer, and in view of the present status of this area the clinical studies are indicated by a question mark in Table 3.

Discussion

It has been established that chemicals that are carcinogenic in man will produce a similar cancer in a variety of animal species. In view of this correlation, chemicals and drugs can be evaluated in animals to predict potential human carcinogenicity. The suspected carcinogen should produce an effect that is not limited to one (or two) species of animals (Table 1); indeed, based on a DNA mechanism we would expect to obtain a similar organ response in most of the species tested. Secondly, in appraising carcinogenicity the magnitude of the response should also be considered; it is usually not appreciated that the known carcinogens produce a positive organ-specific response in a high proportion of the test animals (Table 2).

Research papers on hormones often state that oestrogens have been shown to be carcinogenic in animals, that oestrogens induce tumours in five species of animals, or that oestrogens are hormonal carcinogens. Although such statements have some validity when applied to specific animal studies, they are in general based on a lack of understanding of the effects of oestrogens in animals. For example, it is often stated

that oestrogens induce cancer in mice, but it is not stated or appreciated that the effect is confined to male mice from specific inbred strains that possess a mammary tumour virus (Drill, 1981). The effect of oestrogens on the mammary gland, uterine cervix or the uterine endometrium of different animal species does not demonstrate a carcinogenic profile. Although oestrogens may produce an organ-specific response in the mouse or rat (Table 3), they do not produce the interspecies effect characteristic of a carcinogen. The effect of oestrogen is strain- and species-dependent. Also, when a positive response is obtained only a small proportion of the treated animals are affected, a response that is not typical of a carcinogen.

Clinical data do not demonstrate a relationship between oestrogen administration and the occurrence of breast or cervical cancer, agreeing well with the absence of a carcinogenic profile in animal studies. The clinical appraisal of oestrogens and endometrial cancer is still in progress and in view of the conflicting data that have been obtained, a definite conclusion cannot presently be drawn.

It is generally accepted that the induction of cancer by chemicals involves the covalent binding of the chemical or its metabolites to DNA. Thus, an estimate of carcinogenic potential can be made by procedures that are not dependent on tumour induction. In a highly significant study Lutz & Schlatter (1979) measured the covalent binding of various chemicals to DNA in rat liver. They demonstrated that very potent hepatocarcinogens such as aflatoxin B₁ or M₁ have a high covalent binding index (CBI). Benzene, which does not induce liver tumours gave a very low CBI, and oestrone and ethinyloestradiol gave a CBI slightly less than that of benzene, essentially a negative result.

Oestrogens are necessary for the growth and function of the female reproductive organs and to the extent that they produce functional organs in women they lay the groundwork for the development of cancer. As such they are not carcinogens, but they develop the organs to a functional state that is responsive to carcinogenic influences. The oestrogens have at times been called cocarcinogens or facultative carcinogens, but such terms are not entirely satisfactory; perhaps the oestrogens are best described as having a *non-specific permissive action*.

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(Metzler & McLachlan, 1981). Both the epoxide itself and two rearrangement products could be seen in the gas chromatogram, in a pattern which was also obtained when synthetic DIES-2,3-oxide was gas-chromatographed. Therefore, DIES must be considered to be oxidized at the aromatic ring and at the olefinic double bond. There was no indication of aliphatic hydroxylation.

The oestrogenicity of E.E-DIES is about the same as that of E-DES (Dodds *et al.* 1939). Again, no information is available so far about the foetotoxicity and genotoxicity of this compound.

Conclusion

Current evidence shows that the synthetic oestrogen, DES, is metabolized to a variety of oxidative metabolites along several pathways involving reactive intermediates. This fact, and a positive response in several assays, indicating genetic damage, raise the possibility that metabolic activation may be involved in the carcinogenic effect of DES. The exact mechanism(s) accounting for the organ-directed carcinogenicity of DES is not known, but several possibilities are conceivable (Metzler & McLachlan, 1979). Examples are the formation of reactive metabolites that retain their oestrogenicity and are thus accumulated in oestrogen target organs, and the preferential formation of reactive metabolites in those organs by specific enzymes. The first possibility can be exemplified by E-DES-3,4-oxide (Fig. 2), which has been found to be highly oestrogenic *in vitro* and *in vitro* (Korach, Metzler & McLachlan, 1978). An attractive hypothesis for the organ-specific activation of DES would be peroxidase-mediated oxidation, since oestrogen-inducible peroxidase activity exists in all organs that depend on oestrogens for growth (Anderson, Kang & DeSombre, 1975).

It appears that certain DES analogues such as HES and E.E-DIES may be useful for clarifying further the role of metabolic activation in the genetic toxicity and carcinogenicity of DES. These compounds are powerful oestrogens but they have different routes of metabolism. It will be of interest to determine whether their effects in the different *in vitro* and *in vivo* systems can reasonably be correlated with certain metabolic pathways.

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