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Thyroid gland neoplasia: non-genotoxic mechanisms

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SUMMARY

There are two basic mechanisms whereby chemicals produce thyroid gland neo[illegible] rodents. The first involves chemicals that exert a direct carcinogenic effect in the thyroid [illegible] and the other involves chemicals which, through a variety of mechanisms, disrupt thyro[illegible] function and produce thyroid gland neoplasia secondary to hormone imbalance. There are important species differences in thyroid gland physiology between rodents and humans which may account for a marked species difference in the inherent susceptibility for neoplasia secondary to hormone imbalance. Thus, it is important to consider mechanism in the evaluation of potential cancer risks. There would be little if any risk for apparently nongenotoxic chemicals that act secondary to hormone imbalance at exposure levels that do not disrupt thyroid function. Further, the degree of thyroid dysfunction produced by a chemical would present a major toxicological problem before such exposure would increase the risk for neoplasia for humans.

INTRODUCTION

An understanding of the mechanisms of chemical induction of thyroid neoplasia was obtained during experimentation in the 1940s and 50s as a result of interest in thyroid cancer during this period. It was recognized that two basic mechanisms were involved one of which was by chemicals that exert a direct carcinogenic effect on the thyroid gland. Thyroid tumors have been produced by a variety of direct acting carcinogenic substances such as the polycyclic hydrocarbons [1,2], 2-acetylaminofluorene [3], dichlorobenz-

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idine [4], and a variety of nitrosamines. The second basic mechanism is the production of thyroid tumors by a variety of regimens that result in a hormone imbalance. Kennedy and Purves [5] found thyroid adenomas in rats fed a diet containing brassica seeds, a naturally occurring goitrogen. Numerous studies have demonstrated that treatment with a variety of anti-thyroid substances (thiourea, thiouracil and their derivatives, and 3-amino-1,2,4-triazole) will result in a high incidence of thyroid tumors in rats [6]. Other substances exerting anti-thyroid effects in rats such as some sulfonamides will also produce thyroid tumors [7].

Although some substances that produce hormonal imbalance may also exert a direct carcinogenic effect on the thyroid, it is well established that hormone imbalance alone can increase the incidence of thyroid tumors in rodents. Many anti-thyroid (goitrogenic) substances known to cause thyroid cancer via hormonal imbalance have shown no evidence of a direct carcinogenic effect.

A consistent mechanism, widely accepted by many investigators, to explain the pathogenesis of thyroid tumors induced in rats treated with anti-thyroid drugs has been described [8,9]. Anti-thyroid drugs initially produce a hormonal imbalance by interfering with thyroid hormone production. A sustained increase in the synthesis and secretion of thyroid stimulating hormone (TSH) occurs via the negative feedback system of the pituitary gland to stimulate thyroid function. Increased TSH stimulation produces a variety of morphological and functional changes in the follicular cell including follicular cell hypertrophy, hyperplasia, and ultimately neoplasia. The sustained excessive level of TSH is considered to be the pathogenic factor responsible for thyroid tumor production under these conditions.

That excessive secretion of endogenous TSH alone (in the absence of any chemical treatment) will produce a high incidence of thyroid tumors has been clearly established by experiments in which rats were fed diets deficient in iodine [10–13] or in which TSH secreting pituitary tumors were transplanted into mice with normal thyroids [14]. Iodine deficient diets are goitrogenic and result in an increased TSH secretion with concomitant high incidence of thyroid tumors. These effects can be reversed by iodine supplementation, thyroid hormone replacement, or hypophysectomy.

Goitrogenic substances or regimens are also powerful promoters of thyroid gland neoplasia after the administration of direct acting carcinogenic substances [15–17].

Thyroid hormone synthesis

The functional unit of the thyroid gland is the follicle which consists of an area of colloid, the storage form of thyroid hormone, surrounded by a single layer of follicular epithelium. The thyroid gland is unique among the en-

docrine glands in that hormone synthesis and storage are essentially extracellular processes that occur at the apical surface of the cell membrane. Thyroid hormone synthesis involves the active transport of iodine into the cell and a peroxidase mediated iodination and coupling of tyrosine residues on thyroglobulin to form thyroid hormone which is stored as colloid until released into the circulation [18,19].

The thyroid follicle and the follicular cell are highly organized and polarized structures. Thyroid hormone synthesis involves reactive biochemical processes, and the organization of its structure and function serves to protect the cell from accidental iodination and cytotoxicity from oxidation products. The thyroid peroxidases appear to be active only at the apical surface of the cell membrane.

Thyroid hormone release involves pinocytosis of colloid by microvilli and digestion by lysosomes to release T_4 and T_3 which are secreted into plasma and bound to plasma proteins for transport to peripheral tissues. The most important aspect of the metabolism of thyroid hormone is monodeiodination to form T_3, the physiologically active form of thyroid hormone. T_4 is also metabolized by glucuronidation and T_3 is predominantly sulfated and both are excreted in urine and bile [20].

Regulation of thyroid function

Thyroid gland function is controlled by the hypothalamus and pituitary gland and is regulated via a negative feedback process in which thyrotropin releasing factor (TRH) from the hypothalamus and TSH from the pituitary are released in response to decreased circulating levels of thyroid hormone.

Another important aspect in the control of thyroid function is autoregulation [21,22,23] via a modulation of the receptor response to TSH. The TSH receptor is extracellular and undergoes a conformational change after binding to TSH. The catalytic unit of the receptor (adenylcyclase) produces cyclic AMP (c-AMP) which functions as a second messenger and mediates all the intracellular effects of TSH including the various differentiated functions of the follicular cell and cell proliferation [24]. The adenylcyclase activity of the TSH receptor is subject to autoregulation and is controlled by a class of iodolipids that attenuates the formation of C-AMP [25,26]. When the iodolipids are low as a result of either iodine deficiency or an inhibition of the organification of iodine, the attenuation of the receptor response is removed and the response to TSH is enhanced.

The physiologic role of this autoregulatory process is most likely to modulate the response of the thyroid to TSH depending upon the supply of iodine. Ingbar [27] has referred to this process as the "iodostat" which is essentially a set point in the follicular cell for the response to TSH.

Since autoregulation requires the enzymatic iodination of lipids, a process

similar to thyroid hormone synthesis, inhibitors of iodination will decrease the iodolipid content and thereby enhance the TSH response. Iodine deficiency [21–23] and chemicals such as propylthiouracil (PTU) and methamidazole [28] thus enhance the TSH response beyond that due to increased TSH alone. In contrast, excess iodine [25] or chemicals that can either supply iodine or perhaps be recognized as a modulator similar to the endogenous iodolipids can attenuate the TSH response by inhibiting the formation of C-AMP.

Species differences in thyroid gland biochemistry and physiology

There are marked species differences in thyroid gland physiology that must be taken into account in an evaluation of species differences in the induction of thyroid gland neoplasia secondary to hormone imbalance. The most obvious species difference between rodents and primates is the lack of thyroid binding globulin (TBG) in the rodent and some other species [29]. TBG is the predominate plasma protein that binds and transports thyroid hormone in the blood. Thyroxine binds to three plasma proteins, TBG, pre-albumin and albumin with binding constants of 10^{-10}, 10^{-7} and 10^{-5}, respectively [30]. The lack of TBG in the rodent with binding affinities of 3 and 5 orders of magnitude greater than albumin and pre-albumin may be one of the more important factors responsible for species differences in thyroid gland function.

The half-life (T1/2) of the thyroxine (T_4) is 12 h in the rat versus 5–9 days in humans and serum TSH is 25 or more times higher in the rodent as compared to man [29]. This indicates a much higher activity in the rodent thyroid gland as compared to the primate; a conclusion which would also be supported by the histological appearance of the thyroid gland. In the primate, the follicles are uniformly large with abundant colloid surrounded by relatively flattened follicular epithelial cells. In contrast, the rodent thyroid has large follicles only in the periphery of the gland, the interior of which is comprised of comparatively small follicles with small amounts of colloid surrounded by a more cuboidal follicular epithelium. If rats are supplemented with exogenous thyroid hormone, the follicles will accumulate colloid, increase in size and the epithelial cells will assume a more flattened appearance. Thus, both the physiologic parameters and the histologic appearance indicate that the rodent thyroid gland is markedly more active and operates at a considerably higher level with respect to thyroid hormone turnover as compared to the primate. It is important to note that these and other differences are in the "orders of magnitude" range.

The incidence of "spontaneous" thyroid gland neoplasia is also markedly different. The Fisher 344 rat exhibits a 2% incidence of thyroid follicular cell neoplasia (1% carcinoma and 1% adenoma) [31] as compared to an incidence

of 0.004% with a range of 0.001–0.016% carcinoma in humans [32,33].

The relative susceptibility of rodents and humans to thyroid neoplasia secondary to hormone imbalance or simple hypothyroidism can be assessed by comparing humans in iodine deficient areas of endemic goiter to rats in these same areas or rats treated with iodine deficient diets. Endemic goiter has affected tens of millions of individuals and although relatively extensive epidemiologic studies have been conducted, a clear etiologic role in thyroid gland neoplasia has not been established [34–36]. In contrast, rodents in areas of endemic goiter [37] or those treated with iodine deficient diets [10–13,38] exhibit a high incidence of thyroid gland neoplasia.

The marked species differences between rodents and primates in thyroid gland physiology, the spontaneous incidence of thyroid gland neoplasia and the apparent susceptibility to neoplasia secondary to simple hypothyroidism support the conclusion that thyroid gland neoplasia secondary to hormone imbalance is species specific. The rodent will exhibit an increase in thyroid gland neoplasia in the presence of mild to moderate increases in TSH and may, in fact, be above an "apparent threshold" for TSH mediated neoplasia. In contrast, no clear etiologic role for hypothyroidism in human thyroid cancer has been established even though chronic hypothyroidism, in the moderate to severe range, has occurred in humans in areas of endemic goiter [34]. Thus the contribution of simple hypothyroidism to thyroid cancer in humans is small at most.

MECHANISMS FOR ALTERED THYROID FUNCTION

Thyroid hormone synthesis, release, transport, cellular uptake, conversion of T_4 to T_3, hormone metabolism and the regulation of these processes by the hypothalamic–pituitary–thyroid axis and autoregulatory processes in the thyroid gland itself is a complex process and provides many ways in which chemicals can interfere with thyroid gland function. Regardless of mechanism, the response to hypothyroidism is similar. The pituitary will release TSH as a compensatory response to stimulate the thyroid to produce more hormone. Chronic stimulation of the thyroid gland by TSH in the rodent leads to a progression of follicular cell hypertrophy, hyperplasia and eventually neoplasia [8,14].

Mechanisms can be either intra- or extrathyroidal. Intrathyroidal mechanisms can involve iodine uptake or hormone synthesis and extrathyroidal can involve effects on hormone metabolism or disposition among other mechanisms. A few important examples are discussed below.

INTRATHYROIDAL MECHANISMS

Hormone synthesis

Two classes of chemicals known to inhibit thyroid hormone synthesis are the thiourelyenes (thiourea, propylthiouracil, methimidazole) and the sulfonamides (sulfadiazine, sulfamethazine). The thiourelynes are considerably more potent in inhibiting thyroid hormone synthesis than the sulfonamides, however, sufficiently high dosages of many sulfonamides are goitrogenic in the rodent [7,39–41]. Although the goitrogenicity of the sulfonamides has been known since 1941, only a few have been tested for carcinogenicity. Sulfamethoxazole at dosages of 50 mg/kg/day or more is goitrogenic and produced thyroid neoplasia in rats within 50 weeks of treatment. Sulfamethazine produced goitrogenic effects and thyroid neoplasia in rats at dosages of 600 ppm or more and in mice at dosage of 4800 ppm [42–45]. Sulfisoxazole which is only weakly goitrogenic in rodents did not produce thyroid gland neoplasia at dosages up to 400 mg/kg/day in rats or 2000 mg/kg/day in mice treated for two years [46].

The various sulfonamides that have been tested have no apparent genotoxic effects and the observed thyroid gland neoplasia using goitrogenic dosages is considered to be secondary to hormone imbalance.

Species specificity in the goitrogenic effects of sulfonamides

The goitrogenic effects of the sulfonamides in rodents has been known since 1941. It was also observed that while some species (including rats, mice, hamsters, dogs and swine) were sensitive to the goitrogenic effect of the sulfonamides, no goitrogenic effect was observed in other species (including chickens, guinea pigs or primates) [7,40,41].

In the primate, no effect on thyroid function or morphology was observed in the rhesus monkey treated with sulfamethoxazole [7] for one year at dosage up to 300 mg/kg/day or in cynomolgus monkeys treated for four weeks with sulfmonomethoxine [41] at dosages up to 300 mg/kg/day. In humans, no clinically significant effects on thyroid function have been observed with sulfonamides at therapeutic dose levels [47]. Although Cohen and co-workers [48] observed a small decrease in serum thyroid hormone values in patients treated with cotrimoxazole, no increase in TSH was observed. In addition, no abnormalities in thyroid function were observed in a group of young patients receiving chronic treatment with cotrimoxazole [49,50].

Biochemical basis for species differences

Takayama and co-workers [41] studied the species difference between rats and monkeys using propylthiouracil (PTU) and sulfamonomethoxine

(SMM). The effect of these compounds on thyroid gland function (in vivo) and their ability to inhibit thyroid gland peroxidase from rats and monkeys (in vitro) was assessed. SMM or PTU treated rats exhibited decreased T_3 and T_4 values and markedly elevated TSH values accompanied by follicular cell hyperplasia and increased thyroid gland weight at 30 or 300 mg/kg/day. In contrast, no effect on thyroid function or morphology was observed in monkeys receiving 300 mg/kg/day of either compound.

In vitro there was a marked difference in the inhibition of microsomal thyroid peroxidase (TPO) by SMM depending upon the source of the enzyme. The concentration (IC_{50}) of SMM required to inhibit monkey TPO was approximately 450 times greater than that required to inhibit TPO from rats. With respect to the PTU, the IC_{50} for inhibition of monkey TPO was approximately 50-fold greater than for the rat. Thus there is a quantitative difference between rats and monkeys for inhibition of thyroid function with PTU both in vivo and in vitro. With respect to the sulfonamide, the difference is sufficiently great that this can be considered to be a qualitative species difference in anti-thyroid activity between rodents and primates.

The marked difference in sulfonamide inhibition of rodent TPO as compared to the primate constitutes the biochemical basis for the observed species differences in the goitrogenic effects of sulfonamides. This explains why the sulfonamides are goitrogenic in rodents at relatively low dosages but do not produce effects in monkeys at very high dosages or in humans at therapeutic dosages.

EXRATHYROIDAL MECHANISMS

Monodeiodinases

The conversion of thyroxine (T_4) into the more active hormone triiodothyronine (T_3) occurs in many peripheral tissues and is mediated by a microsomal 5′-monodeiodinase that removes one iodine at the 5′ position in thyroxine [20].

Various iodinated organic compounds such as tetraiodofluoresceine [51], amiodarone [52], and various iodinated radio-contrast media [53], will inhibit the 5′-monodeiodinase and disrupt the conversion of T_4 to T_3. The decrease in serum T_3 values results in a compensatory increase in pituitary TSH. Prolonged treatment with tetraiodofluoresceine at very high dosages will result in a moderate increase in thyroid follicular neoplasia in rodents.

Thyroid hormone metabolism

The effect of chemicals on various aspects of thyroid hormone metabolism have an important impact on thyroid hormone economy in the rodent [54]. The monodeiodinases are quantitatively the most important path in the

disposition of thyroxine. In addition, thyroxine is glucuronidated and T_3 is sulfated and subsequently excreted in bile. Deamination, decarboxylation and cleavage of the ether link occur but are of quantitatively lesser importance [20].

Many chemicals are hepatic microsomal enzyme inducers at high dosages and alter thyroid function in rodents by increasing the hepatic disposition of thyroid hormone [55,56]. Decreased serum thyroid hormone results in a compensatory increase in pituitary TSH which can exert a tumor promoting effect in initiation-promotion models [57] or an increase in thyroid gland neoplasia in two-year carcinogenicity studies [58].

Since small amounts of thyroxine will block the tumor promoting effect of a microsomal enzyme inducer such as phenobarbital, this effect and presumably those observed in two-year studies are secondary to hormone imbalance as opposed to a direct tumor promoting or direct carcinogenic effect in the thyroid gland [58–60].

Clinical significance of extrathyroidal mechanisms

With respect to the chemicals mentioned, tetraiodofluoresceine produced only mild effects on thyroid function in humans at very large multiples of the allowable daily intake [61]. Chronic exposure to anticonvulsant drugs, many of which are enzyme inducing at therapeutic dosages, result in only mild changes in thyroid function (moderately decreased T_4 with normal T_3 and TSH values and a normal TSH response to administered thyrotropin releasing hormone (TRH)) [62]. These changes are not clinically significant.

Although neither inhibition of the monodeiodinase or induction of thyroid hormone disposition represents a species specific effect, these effects need to be considered in the context of the degree of hypothyroidism that will be produced under human use and exposure conditions and the likelihood that this, or any degree of simple hypothyroidism, will present an increased risk for thyroid neoplasia in humans.

Extrapolation

Because of marked species differences in thyroid gland physiology and apparent susceptibility to hypothyroidism, the rodent is an inappropriate model for the extrapolation of cancer risk to man for chemicals that operate secondary to hormone imbalance. On the other hand the rodent will be very sensitive to the action of "direct acting carcinogens". However, even in this case, all other factors being equal, the rodent model is likely to be conservative and overestimate risks for species with different thyroid gland function because of the strong promoting effect of high levels of TSH.

CONCLUSION

Chemicals can alter thyroid function and result in hypothyroidism through a variety of intrathyroidal and extrathyroidal mechanisms. Many of these chemicals will cause thyroid hormone imbalance and neoplasia in rodent carcinogenicity studies. Although in most cases there will not be an alteration of thyroid function under human use or exposure conditions, these mechanisms, with a few notable exceptions, do not represent a species specific effect.

Nevertheless, chemicals that produce thyroid neoplasia in rodents secondary to simple hypothyroidism can be considered to be species specific in that simple hypothyroidism in humans, even in its moderate to severe form, is not an established etiologic factor for human thyroid cancer. The contribution of simple hypothyroidism to human cancer is thus small at most. The degree of hypothyroidism as a result of chemical exposure, would present a major toxicological problem before such exposure presented an increased risk for neoplasia.

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