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Alimentary tract and pancreas

Association of long lasting unsurmountable histamine H_2 blockade and gastric carcinoid tumours in the rat

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SUMMARY The oral administration of loxtidine, a potent histamine H_2-antagonist, to a total of 378 rats at doses of 50, 185, or 685 mg/kg/day for 116 weeks resulted in the late formation of carcinoid tumours of the gastric fundus. The first such tumour was detected after 712 days of treatment. There was no dose related response; 11 rats at the low level of treatment were affected, 12 at the intermediate and 11 at the high. Twenty seven females but only seven males were affected. No gastric tumours were found in the 228 controls. There is no evidence that loxtidine acts as a direct carcinogen and it is suggested that the tumours were the result of prolonged achlorhydria produced by a potent unsurmountable histamine H_2 receptor antagonist.

The fact that spontaneous gastric cancer is very rare in the rat[1–4] renders it a suitable species in which to test the propensity of new medicines to stimulate malignant changes in this organ. That this is so is seen by the response to N-methyl-N'-nitro-N-nitrosoguanidine[5] (MNNG) where well differentiated adenocarcinomas of the antral/pyloric region are commonly seen. Tiotidine, a histamine H_2-antagonist, gave a similar response[6] and another H_2-blocker, SKF93479, produced, after 12 months, hyperplasia and hyperkeratosis of the forestomach with penetration of the muscularis mucosae.[7]

Unlike ranitidine and cimetidine which are competitive, surmountable histamine H_2-antagonists, loxtidine is non-competitive and unsurmountable.[8] The present communication describes the pathological findings in a study during which rats were given loxtidine for the span of their natural lives.

Methods

ANIMALS

Loxtidine was administered at dose levels of 50, 185, and 685 mg/kg/day in the diet* to Charles River CD rats. There were 63 males and 63 females in each test group and 114 males and 114 females in the control group. It was originally planned to continue treatment until the groups reached 20% survival levels.

Plasma concentrations of loxtidine were measured by the method of Harrison *et al.*[9] The animals were frequently inspected for signs of illness and also palpated to detect swellings. Each rat dying or killed before the termination of the experiment was subjected to a careful post mortem examination. Particular attention was given to the stomach which was opened along its greater curvature so that all parts of its surface could be examined. Any abnormal discolouration, depression or elevation was photographed. Standard sections of all stomachs were prepared to include representative samples of squamous region, and the areas of the cardiac, fundic and pyloric glands. In addition all gastric lesions noted at autopsy were identified and sections prepared from the affected areas.

Representative portions of all the organs listed below were also examined histologically: skin and mammary gland, thyroid, trachea, oesophagus, lung, heart, thymus (when present), pancreas, spleen, stomach, small intestine, large intestine, lymph node, salivary glands, liver, adrenals, kidneys, bladder, uterus, prostate, gonads, eye, brain, pituitary. In addition sections were prepared from any tissue showing a macroscopic change.

On day 756 one female rat from the low dose group was killed because of a large mammary tumour. On opening the stomach a round swelling (11×9 mm) was found in the fundic gland area (Fig. 1). It was subsequently found to be histologically

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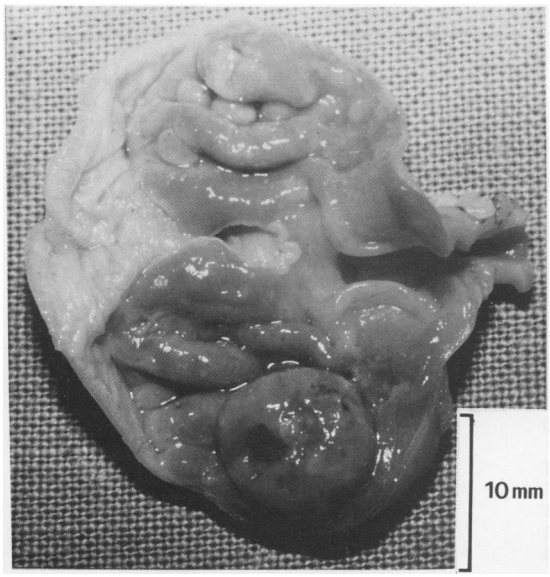

Fig. 1 *Large tumour of the fundic glandular portion of the stomach from a rat given 50 mg/kg loxtidine daily for 756 days.*

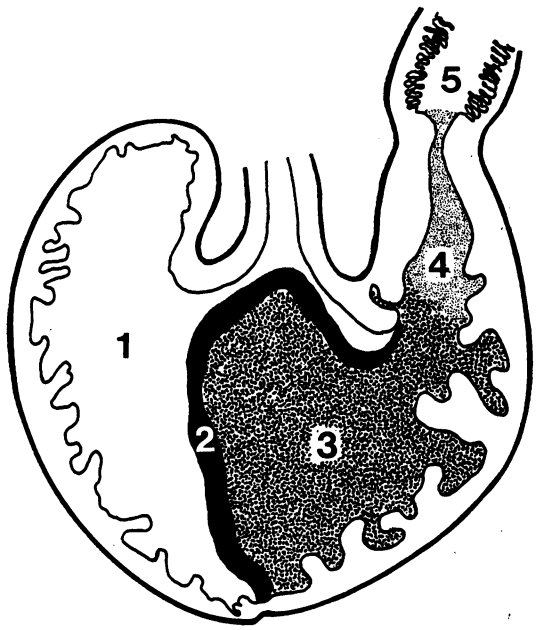

Fig. 2 *Diagram of the rat stomach (LS). 1 squamous non-glandular area, 2 area of cardiac glands, 3 area of fundic glands, 4 area of pyloric glands, 5 duodenum. (After Hebel and Stromberg[10]).*

quite unlike the gastric tumours hitherto produced experimentally in rats. As a result of this finding and as the study was already of 26 months duration it was decided to kill the remaining rats during weeks 115 and 116.

These rats were subjected to the same stringent necropsy. Sections were prepared and examined from all parts of the stomach, the nomenclature used for the regions being a slightly modified version of that of Hebel and Stromberg[10] (Fig. 2). Sections were also prepared from any other tissue showing macroscopic change.

All tissues were fixed in 10% neutral buffered formalin. In addition to standard haematoxylin and eosin, the following staining techniques were used on representative samples: phosphotungstic acid haematoxylin (PTAH), phloxine tartrazine, periodic acid Schiff, Lead haematoxylin, van Gieson, Masson (Singh modificaiton) and Diazo methods for argentaffin granules, Grimelius[11] and Bodian protargol[12] methods for argyrophil granules.

Peroxidase anti-peroxidase (PAP) techniques[13] were used using the following specific antisera: rabbit anti-human gastrin*, rabbit anti-human somatostatin*, rabbit anti-human prealbumin*, rabbit anti-bovine neurone specific enolase*, rabbit anti-porcine pepsin†, monoclonal anti-human serotonin (5HT)‡.

For electron microscopy samples of formalin-fixed tumours were postfixed in phosphate buffered 1% osmium tetroxide and processed into Epon resin. Ultra-thin sections, 60–90 nm thick, were prepared and examined using an AEI 801 transmission electron microscope.

Results

The treated rats remained in good general condition, but their weight gain was less than that of the controls which became obese. At 109 weeks the percentage of each group surviving was as follows: control 32 males, 25 females; low dose 36 males, 46 females; intermediate dose 60 males, 41 females; high dose 65 males, 65 females.

Appreciable plasma concentrations of loxtidine were demonstrated in all treated groups: low 1100–1400 ng/ml, inter 5000–6000 ng/ml, high 20000–28000 ng/ml.

An overall summary of tumours found in all the rats is given in Table 1. A variety of tumours was seen. With the exception of those found in the

* Dakopatts, Mercia Brocades
† Calbiochem
‡ Serotech

Table 1 *Number of rats showing specified tumours at given sites*

	Male				*Female*			
Group	*C*	*L*	*I*	*H*	*C*	*L*	*I*	*H*
Daily dose of loxtidine mg/kg	0	50	185	685	0	50	185	685
Pituitary								
Adenoma	62	32	27	22	84	41	47	25
Mammary gland								
Fibroma	0	1	0	1	3	0	2	0
Fibroadenoma	0	0	0	0	21	13	11	4
Adenoma	0	0	0	0	11	6	8	3
Adenocarcinoma	1	0	0	1	15	10	5	3
Stomach								
Fundic carcinoid	0	2	4	1	0	9	8	10
Squamous papilloma	0	0	1	0	0	0	0	0
Small intestine								
Leiomyoma	0	0	0	0	1	0	0	0
Leiomyosarcoma	0	0	0	1	0	0	0	0
Soft tissue								
Angioma	0	0	1	1	0	0	0	2
Angiosarcoma	0	0	1	0	0	0	0	0
Fibroma	8	7	1	1	0	3	0	0
Fibrosarcoma	2	0	1	1	0	2	0	1
Lipoma	3	2	1	0	3	0	1	1
Adrenal								
Phaeochromocytoma	7	4	2	0	0	1	1	2
Malignant ,,	0	0	1	0	1	0	0	0
Cortical adenoma	2	0	1	0	6	2	1	1
Thyroid								
Adenoma	8	1	1	5	3	2	3	2
Adenocarcinoma	2	2	0	0	2	0	0	1
Lymphoreticular								
Sarcoma	8	7	4	4	1	1	2	1
Pancreas								
Islet cell adenoma	11	5	2	4	4	0	1	1
Exocrine adenoma	0	0	0	0	0	0	1	0
Skin								
Basal cell tumour	2	0	0	0	0	0	0	0
Basal cell carcinoma	0	0	0	1	0	0	0	0
Trichoepithelioma	2	0	0	0	0	0	0	0
Keratoacanthoma	1	1	0	0	0	0	0	0
Squamous papilloma	2	1	0	0	0	0	0	0
Squamous cell carcinoma	0	0	1	0	0	0	0	0
Sebaceous adenoma	1	0	0	0	0	0	0	0
Sebaceous adenoma (Zymbal's gland)	0	0	0	1	0	0	0	0
Testis								
Interstitial cell tumour	3	2	5	2	—	—	—	—
Brain								
Astrocytoma	1	1	1	0	1	0	0	0
Granular cell tumour	1	1	0	0	1	0	0	1
Liver								
Adenoma	2	0	0	1	0	1	0	0
Adenocarcinoma	0	2	2	0	0	0	0	0
Kidney								
Cortical adenoma	0	0	1	0	0	0	0	0
Carcinoma	1	1	0	0	0	0	0	1
Squamous carcinoma	0	0	1	0	0	0	0	0
Ovary								
Granulosa cell tumour	—	—	—	—	3	2	1	1
Uterus								
Squamous cell carcinoma	—	—	—	—	1	2	0	0
Fibroma	—	—	—	—	0	1	0	0
Accessory sex glands								
Carcinosarcoma	1	0	0	0	0	0	0	0
Preputial gland adenoma	0	0	0	0	0	0	1	0
Prostate adenocarcinoma	0	0	1	0	—	—	—	—

(Continued overleaf)

Table 1 *(Continued)*

Group	Male				Female			
	C	*L*	*I*	*H*	*C*	*L*	*I*	*H*
Head								
Squamous carcinoma (jaw)	0	0	0	0	0	0	1	0
Carcinoma (orbital tissue)	0	0	0	1	0	0	0	0
Salivary gland								
Adenoma	0	0	0	0	1	0	0	0
Mixed tumour	0	0	0	1	0	0	0	0
Parathyroid								
Adenoma	0	0	0	0	1	0	1	0
Blood								
Granulocytic leukaemia	1	0	0	0	0	0	0	0
Lymphocytic leukaemia	1	0	0	0	0	0	0	0
Others								
Mesothelioma	0	0	1	0	0	0	0	0
Neurofibrosarcoma	0	0	0	0	0	1	0	0
Sarcoma (thorax)	0	1	0	0	0	0	0	0
Thymoma	0	0	0	1	0	0	0	0
Totals	134	74	62	50	163	96	95	60

NB. The control groups contain 114 rats of each sex and the treated 63 of each sex.

glandular stomach, however, all were of types commonly encountered in rats of this strain.

NEOPLASTIC STOMACH CHANGES

Some animals killed or dying before the termination of the experiment had small pale raised areas restricted to the mucosa of the fundic area (Fig. 3). These were often multiple and ranged in size from 1 to 11 mm, the large ones often showing erosions.

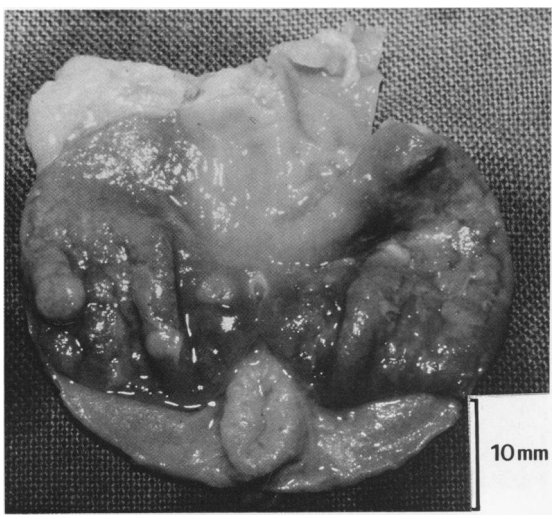

Fig. 3 *Multiple pale raised areas in the fundic glandular region of the stomach. From a rat given 685 mg/kg loxtidine daily for 798 days.*

They are summarised in Table 2.

Histological examination of the altered areas revealed a progressive sequence of changes, the earliest occurring in the gastric glands of the fundus. The mucus secreting cells forming the foveolae were unchanged but, deeper in the gland, where normally parietal cells and chief cells are found, the epithelial layer consisted largely of very pale staining, undifferentiated cells. The basic architecture of the gland was often unaltered, it being lined by a single layer of the pale cells, but in other glands the pale cells had proliferated to form small nests, thereby disrupting the gland architecture (Figs. 4, 5). From the study of these early changes it was apparent that this intramucosal proliferation progressed to form small tumours.

The early smaller tumours were all composed of undifferentiated cells forming aggregates not sharply demarcated from the normal glandular epithelium of the fundic area. There was a remarkable consistency in cellular morphology: the cells were larger than either chief or parietal cells and had large amounts of poorly stained cytoplasm often with a foamy appearance; stains for mucus were negative and cellular outlines were often indistinct. The nuclei were of uniform size and shape, large, pale and open with a prominent, usually central, nucleolus and delineated by a fine line of marginal chromatin.

In areas where changes had progressed further, nests of pale cells were also present in or below a distinct muscularis mucosae. Such an appearance was taken to intimate early malignancy. The final stage of the progression to unequivocal malignancy

Table 2 *Chronological appearance of lesions of the glandular stomach (fundus)*

Animal (no)	*Sex*	*Dose group*	*Day*	*Gross description*	*Microscopic appearance*
18432	M	I	657	Pale raised rounded area (4 mm)	IMP
18803	F	I	676	Pale raised swelling (3 mm)	IMP
18369	M	L	699	Small swelling (3 mm)	IMP
18396	M	I	712	Smooth round while nodule (1 mm)	Early carcinoid
18800	F	I	715	3 pale raised areas (3 mm)	IMP
18607	M	L	735	Raised area (2 mm)	IMP
18437	M	I	740	Nothing abnormal seen	IMP
18751	F	L	756	Large eroded swelling (11×9 mm)	Malignant carcinoid
18717	F	I	782	2 pale slightly rounded areas (3 mm)	IMP
18798	F	I	784	Several raised rounded areas (2–5 mm)	Early carcinoid
18490	M	L	791	Pale raised rounded areas (up to 5 mm)	IMP

IMP=Intramucosal proliferation.

was typified by the spread of pale staining cells through an indistinct muscularis mucosae (Fig. 6) with tumour cells in submucosal lymphatics (Fig. 7) and in some cases, invasion of blood vessel walls. The full malignant potential was confirmed by the finding, in one rat only, of a large deposit of tumour

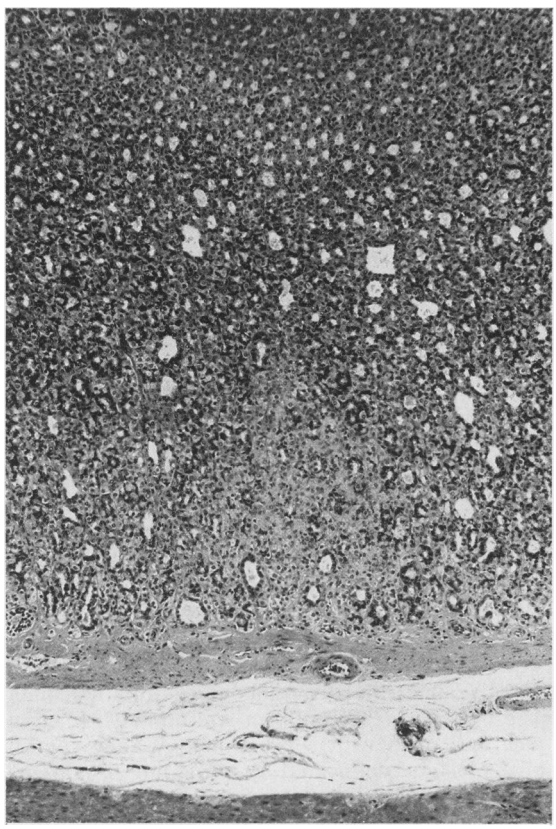

Fig. 4 *A wedge shaped accumulation of pale-staining cells confined to the mucosa and locally disrupting the glandular pattern. From a rat given 50 mg/kg loxtidine daily for 804 days. Haematoxylin and eosin×100 (original magnification).*

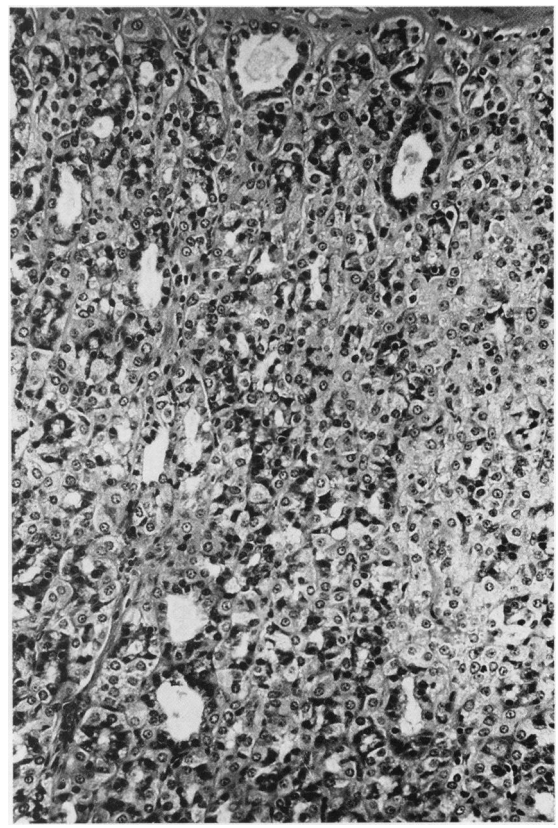

Fig. 5 *Detail of Fig. 4. The gastric glands to the left are normal but on the right there are collections of uniform, undifferentiated cells with pale cytoplasm. Haematoxylin and eosin×250 (original magnification).*

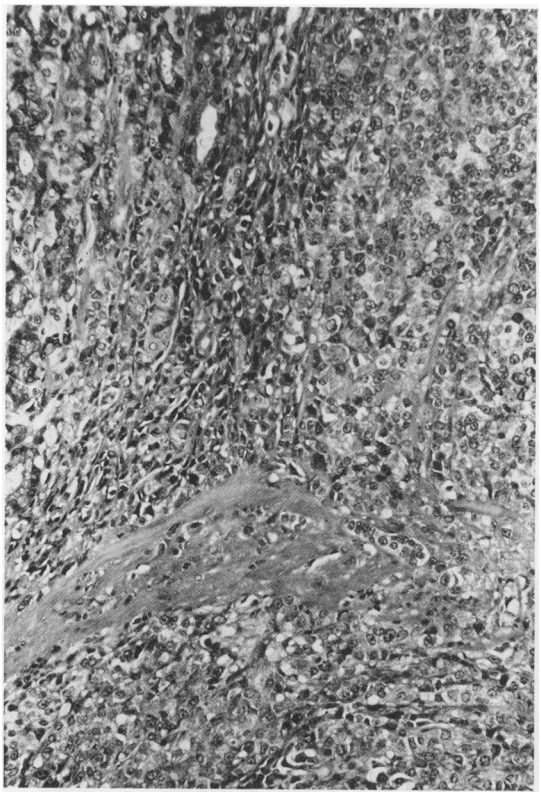

Fig. 6 *A carcinoid tumour extending through the muscularis mucosae into the submucosa. From a rat given 50 mg/kg loxtidine daily for 799 days. Haematoxylin and eosin×250 (original magnification).*

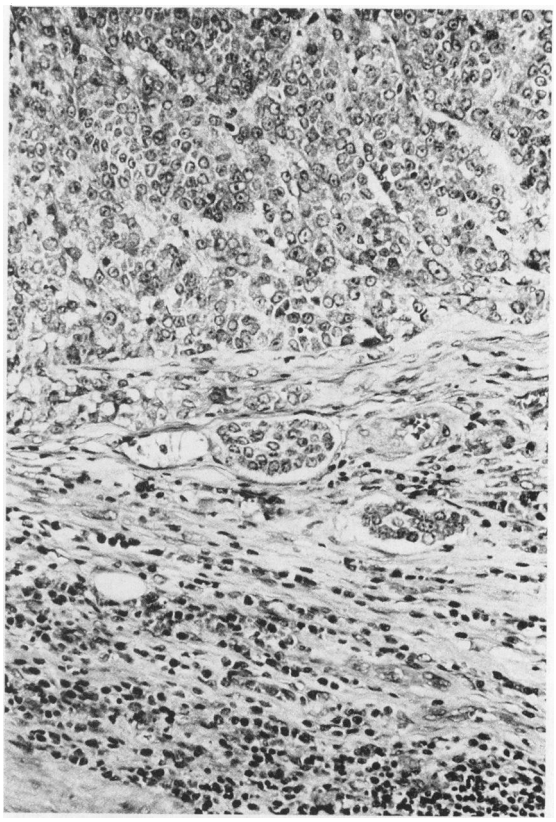

Fig. 7 *Tumour cells in submucosal lymphatic vessels. From a rat given 185 mg/kg loxtidine daily for 796 days. Haematoxylin and eosin×300 (original magnification).*

cells substantially replacing a draining lymph node. No metastatic deposits were seen in any other tumour-bearing animal.

In general, the tumours were composed of pale cells arranged in a solid fashion but in some of the larger ones tumour cells were arranged in clumps surrounded by connective tissue (Fig. 8). The larger tumours did show, in some places, differentiation into a glandular form resembling that seen in some carcinoids. This was particularly noticeable in the metastatic deposit in the lymph node (Fig. 9).

When all the remaining rats had been killed and histologically examined, the full extent of the changes could be appreciated (Table 3). No obvious dose relationship could be seen but many more females than males were affected.

In many rats showing histological evidence of early tumours or intramucosal proliferation, no gross lesions were noted. Thus, it is conceivable that, unless these early changes were of widespread

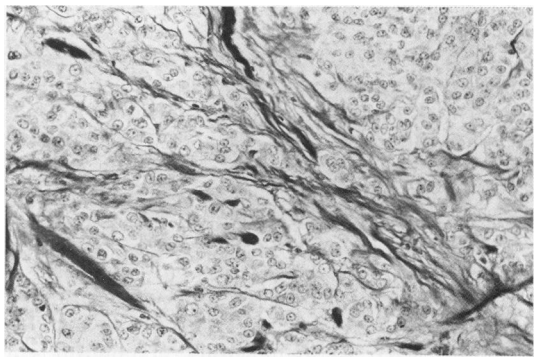

Fig. 8 *Nests of tumour cells delineated by collagen fibres; area of a submucosal tumour from a rat given 685 mg/kg loxtidine daily for 799 days. Van Gieson×400 (original magnification).*

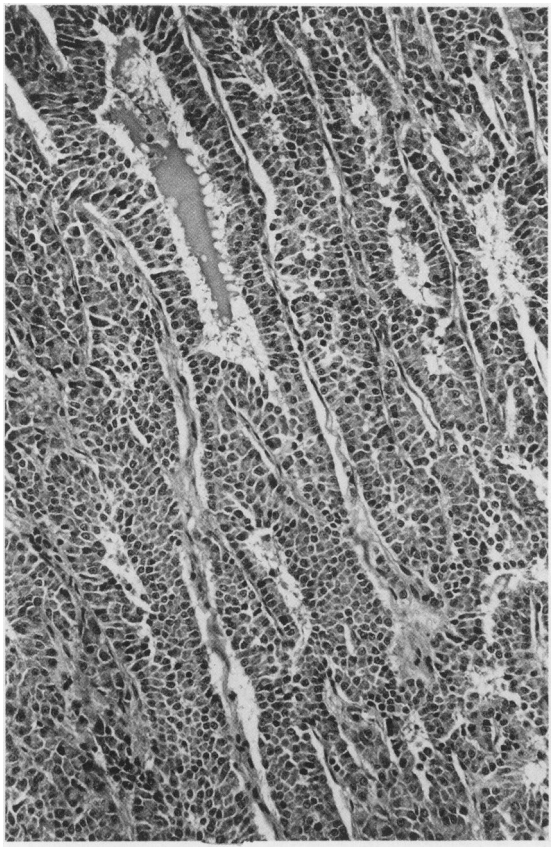

Fig. 9 *Lymph node with metastatic deposit showing gland-like organisation. Same rat as in Fig. 7. Haematoxylin and eosin×250 (original magnification).*

occurrence, they might well be missed. Unequivocally malignant tumours were always visible macroscopically.

No significant differences were seen with respect to the gastric squamous area of the control and treated rats. In the glandular stomach all changes were restricted to the fundic area; no changes were seen elsewhere, either macroscopically or microscopically in any rat. No stomach changes suggestive of neoplasia were seen in the control rats.

The tumours bore a resemblance to the gastric neuroendocrine tumour of *Mastomys nataliensis*.[14] All the 34 tumours found in the loxtidine treated rats were consistently negative by the methods commonly used to demonstrate argentaffin granules. The Bodian protargol method for argyrophilic granules was similarly negative in all 34 tumours but the Grimelius technique was able to distinguish sparse granularity but in only four of the tumours, all of which were small (Figs. 10 and 11).

Tumour cells were consistently negative using the specific antiserum techniques for possible secretory products. Good cross-reactivity between the antisera and rat tissue could be demonstrated by the strong positive staining of neuroendocrine cells of the pyloric mucosa in the case of antigastrin or antisomatostatin, and the finding of neural tissue cells exhibiting strong reactivity to neurone specific enolase. Although it was not possible to be certain that the antiserotonin and antiprealbumin cross reacted with rat tissue antigens, the reagents gave positive reactions in sections of human carcinoid. The appropriate negative controls were always included to eliminate any non-specific reactivity.

Recently Rode and his colleagues[15] showed the presence of protein gene product 9·5 (PGP 9·5) as a valuable additional probe in the diagnosis of neuroendocrine tumours. Using this technique, Dr J

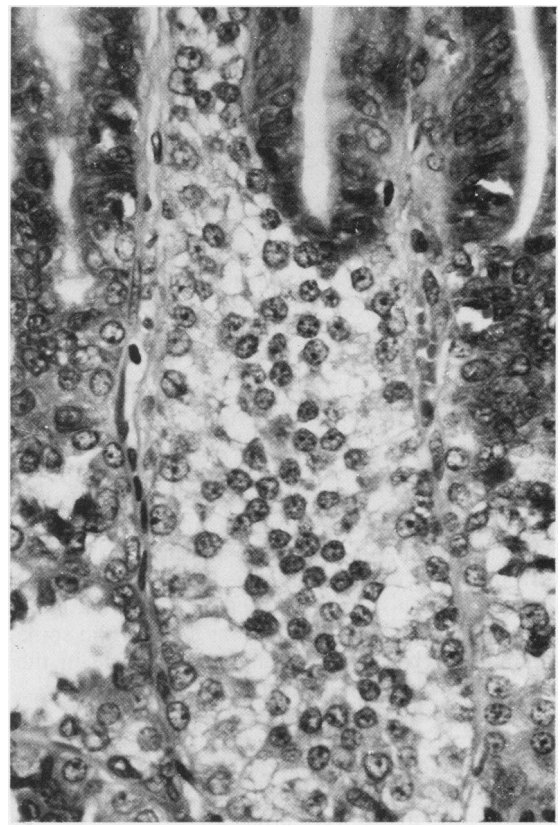

Fig. 10 *Intramucosal nest of cells situated in lamina propria between foveolae. From a rat given 685 mg/kg loxtidine daily for 804 days. Haematoxylin and eosin×625 (original magnification).*

Table 3 *Macroscopic appearance of hyperplastic and neoplastic lesions of the fundic stomach*

			Macroscopic appearance of lesions				
			Pale rounded areas in fundus (mm diameter)			*Lesions not*	
Dose group	*Sex*	*Histological Diagnosis*	*11–15*	*6–10*	*1–5*	*visible*	*Totals*
Control	M & F	MC	0	0	0	0	0
		EC	0	0	0	0	0
		IMP	0	0	0	0	0
Low	M	MC	0	0	1	0	1
		EC	0	0	1	0	1
		IMP	0	0	6	1	7
	F	MC	1	0	1	0	2
		EC	0	0	4	3	7
		IMP	0	0	1	2	3
Inter	M	MC	1	0	2	0	3
		EC	0	0	1	0	1
		IMP	0	0	2	2	4
	F	MC	0	0	0	0	0
		EC	0	0	7	1	8
		IMP	0	0	5	3	8
High	M	MC	0	0	0	0	0
		EC	0	0	1	0	1
		IMP	0	0	1	4	5
	F	MC	0	1	0	0	1
		EC	0	0	1	8	9
		IMP	0	0	1	5	6

IMP=Intramucosal proliferation, EC=Early carcinoid, MC=Malignant carcinoid.

Rode was able to show the presence of this marker in our rat sections, both in areas showing intramucosal proliferation and in larger tumour deposits. Similar results were obtained by him for neurone specific enolase using antiserum raised in rabbits to the human immunogen. This antiserum gave better results than the commercial preparation used by us.

Transmission electron microscopy was carried out on three of the larger tumours whose size had permitted some formalin fixed material to be stored in the wet condition. Examination of sections showed that in approximately 95% of the cells small membrane bound electron dense granules were present (Fig. 12). Their number in each cell varied. Some cells showed numerous granules whilst others only showed three or four small granules. Measurements made on 30 cells showed that the granules ranged in diameter from 80–420 nm. Such granules are charcteristic of neuroendocrine cells. The cells showed a prominent rough endoplasmic reticulum and many free ribosomes, and the nuclei sometimes exhibited invaginations. In many cells small bundles of cytoplasmic microfilaments were seen.

OTHER TREATMENT RELATED STOMACH CHANGES

A further change was seen in a high proportion of treated rats but again this showed no dose relationship either in incidence or severity. In affected animals, the apical cytoplasm of cells lining the basal two thirds of the gastric glands was packed with vividly eosinophilic granules. Such cells contained a basally placed nucleus surrounded by basophilic cytoplasm. The extent of this change varied from single affected glands to large groups of adjacent affected glands; normal parietal and chief cells were often present alongside the eosinophilic cells. Affected glands could be found in normal mucosa or adjacent to areas of mucosal damage. There was no association with the neoplastic or proliferative areas previously described. The earliest observation of these cells was made in a rat which had been on test for 498 days and careful search of the stomachs from control rats revealed only solitary examples of such eosinophilic cells in very few animals.

These cells also stained orange with phloxine tartrazine, and PTAH showed many purple granules in the cytoplasm. They appeared not to be altered chief cells because they could not be stained for pepsinogen (PAP reaction), although pepsinogen granules were demonstrated in quantity in surrounding chief cells. The cells did not resemble rat ileal Paneth cells in their staining properties and electron microscopy failed to show an obvious relationship to any known cell of the gastric mucosa. Such cells have been found in rats fed sulphite[16 17] and in some rats receiving H_2 blockers such as SKF93479 (Bet-

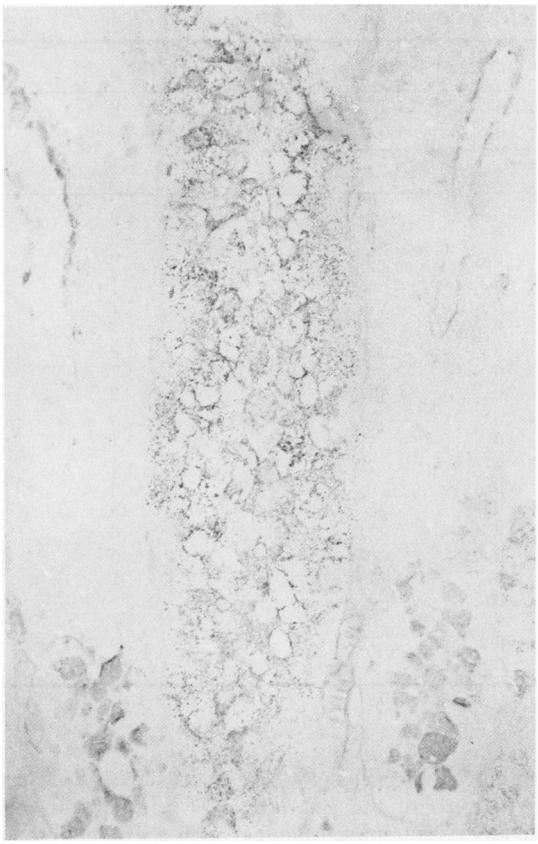

Fig. 11 *Further section of same area as in Fig. 10 demonstrating argyrophil granules within proliferating cells. Grimelius×625 (original magnification).*

ton, personal communication). They were not found in rats given ranitidine for 30 months.[18]

In all other respects the histological appearance of the stomach mucosa in treated rats showed no obvious difference from that seen in control rats.

RELATED STUDIES

Apart from the tumour study, loxtidine was subjected to extensive toxicological testing, including an 18 month study in the rat and a 12 month study in the dog. The compound was well tolerated and caused no treatment related tissue changes at daily levels up to 100 mg/kg in the dog and 500 mg/kg in the rat. Reproductive studies in the rat and rabbit were similarly uneventful.

No detectable genetic toxicity was found in the Ames test, the fluctuation test or in the gene conversion assay either with or without a rat liver enzyme fraction (S9). No mutagenic products were detected following a WHO nitrosation procedure. Loxtidine was also negative in a mouse micronucleus test.

Discussion

Loxtidine, when given to rats for the whole of their natural life span, is responsible for the production of an unusual tumour of the fundic glandular stomach. Electron microscopy of some of the large tumours showed in every case that the tumours contained a large proportion of cells with cytoplasmic granules typical of neuroendocrine cells, thus substantiating a diagnosis of carcinoid tumour. This was further confirmed by the demonstration of specific neuroendocrine markers in both the proliferative lesions and tumours. No specific functional activity could be ascribed to these tumours using specific antibody techniques, and electron microscopy showed a range in granule sizes which made further classification on grounds of granule morphology impossible. In view of the consistency in cellular morphology between the small intramucosal proliferations and the large tumour masses it is reasonable to assume that all lesions were representative of a continuous sequence from neuroendocrine hyperplasia through microcarcinoidosis to carcinoid tumour.[19]

Spontaneous tumours of the glandular stomach are rarely reported in the rat,[1–4] and the occurrence of spontaneous carcinoid is unrecorded. Spontaneous carcinoid tumours and their precursor lesions are well documented for the rodent *Mastomys nataliensis*,[20] however, and they bear a striking resemblance to those seen in loxtidine treated rats. The first carcinomas reliably induced in the rat glandular stomach were produced by the intramural injection of aromatic polycyclic hydrocarbons, in particular 2,7 fluorenylacetamide. A notable observation was made in 1967 when MNNG in drinking water was found to produce tumours in the glandular stomach but not in the forestomach.[5] The use of this substance led to the development of a valuable model for the study of gastric cancer, and valuable model for the study of gastric cancer, and the work has been comprehensively reviewed.[21 22] It is apparent from the many reports available on MNNG-induced carcinomas of the glandular stomach, that the vast majority of tumours are well differentiated adenocarcinomas of the antral/pyloric region.[23] N-methyl-N′-nitro-N-nitrosoguanidine induces, although at a much lower frequency, adenocarcinoma of the small intestine and a variety of tumours in tissues other than the alimentary tract. It is interesting to note, however, that lower doses of MNNG given for a shorter time can induce small numbers of carcinoid tumours of the fundic region, although the method is not as reliable as that for

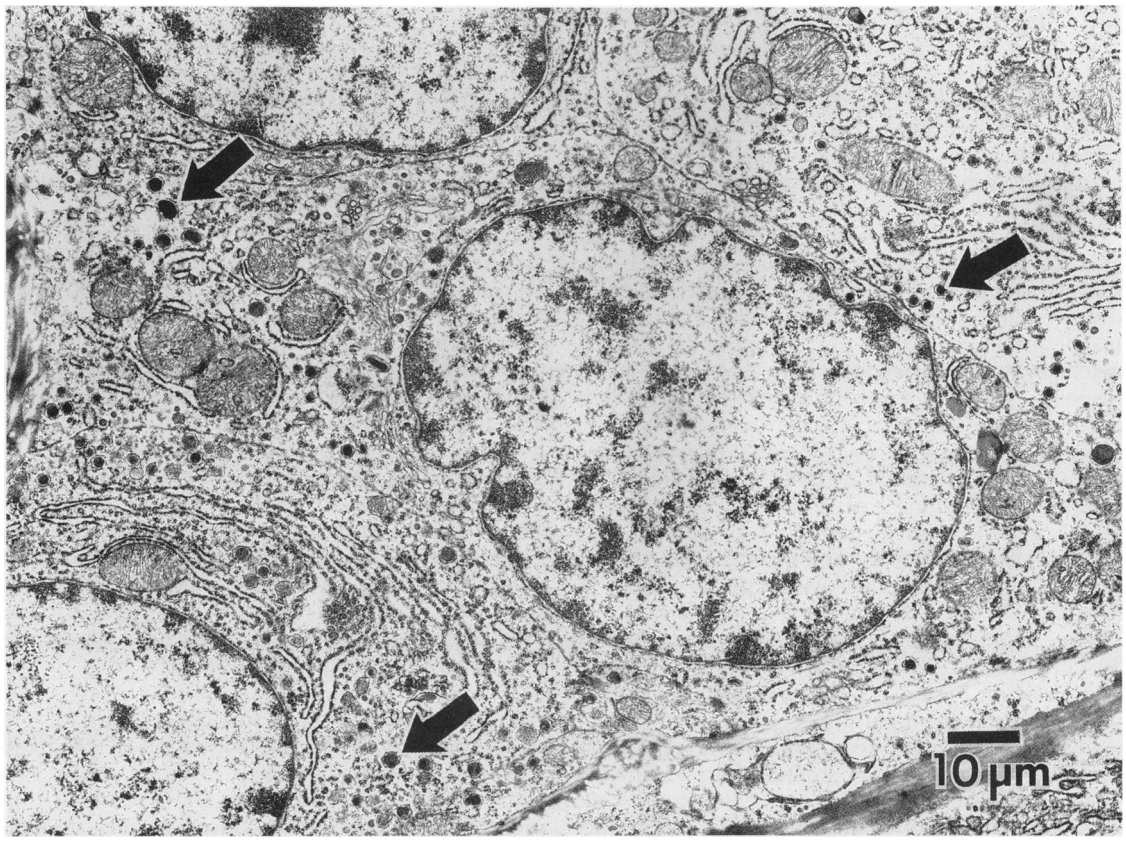

Fig. 12 *Electron micrograph showing parts of four tumour cells. Characteristic carcinoid granules (→) are present in the cytoplasm of all cells. From a rat given 685 mg/kg loxtidine for 799 days.*

producing adenocarcinomas.[24]

Both neoplastic and hyperplastic changes in the rat stomach have been produced following treatment with histamine H_2-antagonists. The first to produce gastric carcinoma in the rat was tiotidine.[6] A total of 17 out of 828 treated rats were found to have dysplastic and malignant changes, located primarily in the pyloric region, similar to those produced by MNNG[23] and some of these changes were detected after only six months treatment. Another histamine H_2-antagonist, SKF 93479, was responsible at one year for the induction of hyperplasia and associated hyperkeratosis of the non-glandular stomach of the rat.[7] Penetration of the muscularis mucosae was seen in two animals. The results of two years treatment are awaited. The fundic tumours seen in association with loxtidine bear no resemblance to the well differentiated adenocarcinomas seen with MNNG or tiotidine.

There is no doubt that loxtidine induced tumours are treatment related but the following facts lead to the conclusion that they are not produced by a direct carcinogenic effect inherent in the loxtidine molecule. (1) Loxtidine is not a demonstrable mutagen, either with or without metabolic activation; further it is not nitrosated to a mutagenic entity. (2) There is no dose relationship in the incidence of gastric carcinoid tumours. (3) In a previous study, when administered daily for 18 months by gavage to PVG hooded rats at doses as high as 500 mg/kg, loxtidine was without tumorigenic effect. (Blood concentrations of 34000–42000 ng/ml were attained). (4) In the present study the first gastric carcinoid was not detected until 712 days of treatment had elapsed. (5) Loxtidine exerted its tumorigenic effect only on the glandular stomach. (6) The tumours occur in the fundic area and are carcinoids, whereas those associated with known carcinogens affecting the glandular stomach – for example, MNNG,[23] tiotidine,[6] are seen in the pyloric region and are largely

well differentiated adenocarcinomas.

We know of no carcinogen which is not mutagenic and which is not recognised until 712 days of treatment and which thereafter, manifests itself in a non-dose related fashion. Further we know of no gastric carcinogen which produces only fundic carcinoids and which is so precisely organ and site specific.

As it is unlikely that the rat tumours are attributable to loxtidine being a direct carcinogen we suggest that their appearance is a consequence of the unremitting achlorhydria produced by the pharmacological action of the compound. Such a mechanism would explain the lack of a dose response because there is no doubt that the effects on the suppression of acid occurred at all the dose levels studied. In this case we would expect similar tumours to be produced by other agents capable of completely inhibiting gastric acid secretion. As in our experience, such tumours do not become apparent until after very prolonged treatment it is essential that compounds with effects similar to loxtidine be tested for the life span of the rat.

The control of gastric acid secretion is complex and involves interaction of endocrine, neurocrine, and paracrine systems.[25] Unremitting achlorhydria could remove an important feedback mechanism, leading to a significant disturbance of the regulatory processes. This might be reflected in an uncontrolled increase in one or more of those cells involved in the control of acid secretion. The fact that tumours were only found late in the study is probably related to the failure of surveillance mechanisms in the aging rat.

The presence of acid in the antrum has an inhibitory effect on the release of gastrin from the gastric G cells,[25 26] histamine H_2-antagonists have been shown to increase serum gastrin concentrations,[26] and hypergastrinaemia is well documented in cases of achlorhydria associated with atrophic gastritis or pernicious anaemia.[25] In the rat, gastrin causes release of histamine[27] from the ECL cells of the gastric fundus. If these cells were constantly stimulated by sustained high levels of gastrin it might be supposed that a compensatory proliferation of such cells would result, with the possibility of eventual neoplastic transformation. Indeed, neoplastic proliferation of ECL cells in association with hypergastrinaemia has been described in man.[28] Such a hypothesis cannot be substantiated by the results of the present experiment because we have been unable to identify the exact nature of the proliferating cell by immunohistochemical means or by granule morphology seen by electron microscopy. It is, however, quite possible that these cells are the result of dedifferentiation to a more primitive precursor state.[29]

The hypothesis that the carcinoids associated with loxtidine are related to its effect on acid secretion is supported by observations from human patients because in a survey of 42 cases Wilander and his colleagues[30] found that the most frequent clinicopathological correlation was achlorhydria linking pernicious anaemia and gastric carcinoids.

The extrapolation of rodent carcinogenicity studies to the possible clinical use of a new compound in human patients is uncertain. Substances which produce malignant changes in animals, no matter the dose level or the duration of the study are not used clinically unless there is a major benefit for the patients treated. The position with loxtidine is clear. The tumours are malignant and in our view the possible therapeutic advantages are not sufficient to warrant any treatment associated risk. The reason is that cimetidine and ranitidine are effective drugs which, unlike loxtidine, are not carcinogenic in rats. Cimetidine was tested for 24 months (730 days) at doses up to 950 mg/kg/day[31] and ranitidine was subjected to life-span studies lasting from 875 to 903 days, at dose levels up to 2000 mg/kg/day which gave plasma levels as high as 8900 ng/ml.[18]

Daily administration of loxtidine to rats for 116 weeks resulted in the late formation of carcinoid tumours in the fundic region of the stomach. The evidence indicated that the tumours resulted from prolonged achlorhydria produced by unsurmountable histamine H_2-receptor blockade. In view of the late appearance of these tumours, we believe that it is essential for compounds with effects on acid secretion similar to that of loxtidine to be tested for the natural life-span of the rat.

We are grateful to the many people whose efforts ensured that this long term study involving many rats was successfully completed. In particular, we thank Dr Basil Morson who first suggested to us the true nature of this tumour and Dr Jurgen Rode for the immunohistochemical demonstration of its neuroendocrine origin. The skilled help of Sheila Riches and Steve Papworth is also recognised and we thank Doreen Newton and Karen Varley for their preparation of the manuscript.

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