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Effect of Maternal Paraquat Administration on the Pyloric Region of the Developing Rat Stomach

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The effect of paraquat (PQ, 1,1′-dimethyl-4,4′-bipyridium) on the histogenesis and glycoconjugates (GCs) properties of the pyloric region of the stomach in a perinatal rat was examined by histological and histochemical methods. Oral administration of PQ (9 mg/kg per day in 0.2 mL of D.W.) on 7 to 14 days of gestation revealed growth retardation with significant reductions in the length of pyloric gland and their pit. As for histochemical properties of GCs in the pyloric region of the stomach, the PQ-treated rats showed some differences, such as delayed initial appearance of the sulfated GCs and lectin affinities compared with the vehicle group. These different GCs properties in the surface and gastric pit were usually detected in the fetal rats and more prominent and evident differences were revealed in the gland epithelium of the early postnatal rat. These results suggest that maternal PQ administration causes intrauterine growth retardation associated with delayed histogenesis and GCs immaturation of pyloric mucosa in developing rat.

A great number of highly toxic herbicides capable of causing fatal poisonings in man and animals have widely been used in the destruction of noxious weed. Of the herbicides, paraquat (PQ, 1,1′-dimethyl-4,4′-bipyridium) is most commonly used and an effective one. It has been proposed that PQ is a strong generator of superoxide anions, which is able to react with nitric. These highly toxic radicals are extremely reactive with macromolecules and may result in multiple organ injuries involving damage to cell membranes in several species (Stamler et al., 1992; Wang et al., 1992; Patel et al., 1996).

PQ is regarded as a highly embryotoxic agent on amphibian development (Vismara et al., 2000), but lacks any teratogenic effects when administered in pregnant mice or rats (Bus et al., 1975; Selypes et al., 1980). However our previous studies showed that oral administration of PQ to a pregnant rat has a great influence on the differentiation of enteroendocrine cells in the stomach and duodenum of fetal rat '(Choi, 1998). With physiological function in digestion, enteroendocrine cells may also play an important role in differentiation and morphogensis of the gastrointestinal tract (Onolfo and Lehy, 1987).

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Because various cell types and numerous enteroendocriene cells are found in the pyloric region of stomach, PQ may have affect on the ontogenesis and glycoconjugates (GCs) properties in this area. The present study has been undertaken to clarify the effect of PQ on histogenesis and GCs maturation of the pyloric region of the stomach in perinatal rat by histological and histochemical methods.

Materials and Methods

Animals and PQ administration

Female Sprague-Dawley rats from 12 to 15 weeks of age were placed overnight from 18:00 to 8:00 h with males and the presence of sperm in vaginal smear was examined on the following morning. The day on which sperms in vaginal smear was identified was designated as day 0 of gestation. Pregnant rats were orally-administered daily with 9 mg/kg PQ and control ones with the same volume of distilled water from 7 to 14 days of gestation. Four period-groups were used: fetal (17, 19 and 21 days of gestation), suckling (1, 3, 5, 7, and 14 days old), weanling (21 days old) and adults (105 days old) in relation to pre- and postnatal development. Body weight and crown-rump length of developing rats were measured and a comparison of the influence of PQ was made using Student's t-test.

Table 1. Lectins used for identifying carbohydrate residues

Lectin	Source	Major sugar specification	Concentration (μg/mL)
DBA	*Dolichos biflorus*	α-N-acetyl-D-galactosamine	10
SBA	*Glycine max*	α/β-N-acetyl-D-galactosamine	10
PNA	*Arachis hypogaea*	Galactosyl-(β-1,3)-N-acetyl-D-galactosamine	10
BSL-1	*Bandeiraea simplicifolia*	α-D-galactose	10
RCA-1	*Ricinus communis*	β-D-galactose	5
sWGA*	*Triticum vulgaris*	β-N-acetyl-D-glucosamine	5
UEA-1	*Ulex europaeus*	α-L-fucose	10
Con A	*Canavalia ensiformis*	α-D-mannose, α-D-glucose	2
LCA	*Lens culinaris*	α-D-mannose, α-D-glucose	3

* sWGA: succinylated WGA

Histological analysis and histochemistry for GCs

The pyloric region was obtained from perinatal and adult rats and fixed in 10% neutral buffered formalin for 24 h. Tissues were dehydrated in a graded ethanol series and embedded in paraffin. Serial 5 μm thick sections were prepared. For histological analysis, hematoxylin-eosin stain and periodic acid Schiff's (PAS) reaction were used. The depth of pyloric gland and their pit of developing rats were measured. For the general GCs histochemistry, the following staining procedures were used: the PAS reaction for studying neutral GCs, alcian blue (AB) staining at pH 2.5 for the demonstration of acidic GCs, and AB at pH 1.0 and a combination staining of aldehyde fuchsin (AF) at pH 1.7 and AB at pH 2.5 to allow the difference of sulfated and nonsulfated GCs.

For lectin histochemisty, deparaffinized sections were treated with 0.3% methanolic H_2O_2 for 30 min to remove peroxidase remains in tissues. To prevent non-specific immunoreactions, sections were exposed to 1% bovine serum albumin for 30 min at room temperature. Nine different biotinylated lectins purchased from Vector Laboratories Inc. (Burlingame, CA) were used whose concentration and oligosaccharides specification are shown in Table 1.

Sections were incubated with lectins for 18 h at 4℃, and rinsed with phosphate buffered saline (PBS, 10 mM, pH 7.4), and then incubated with an avidin-biotin-peroxidase-complex (Vector Lab.) for 1 h at room temperature. Following PBS rinse, horseradish peroxidase (HRP)-conjugated lectin was visualized by exposure to 0.02% 3,3-diaminobenzidine tetrahydrochloride and 0.01% H_2O_2 in Tris buffer (50 mM, pH 7.6). Sections were rinsed with PBS, distilled water, and counterstained with Mayer's hematoxylin. As controls for the lectin histochemistry, sections were incubated with HRP-conjugated lectins in the presence of 0.2 M inhibitory sugars.

Results

Body weight, crown-rump length measurement, and histological analysis

Measurement of body weight and crown-rump length and histological analysis of the pyloric gland of rats are presented in Figs. 1 and 2. Oral administration of PQ showed some reduction in body weight in the 17- and 21-day fetal rats and 5-day old rats and in crown-rump length in the 17- and 19-day fetal rats with changes in shape of the pyloric gland and their pit. PQ significantly decreased the depth of the pyloric gland and pit, especially in the 21-day fetal rats and 3-day old rats, compared with the vehicle group.

General GCs histochemistry

Results of the histochemical properties of GCs in the pyloric region of the stomach using conventional methods

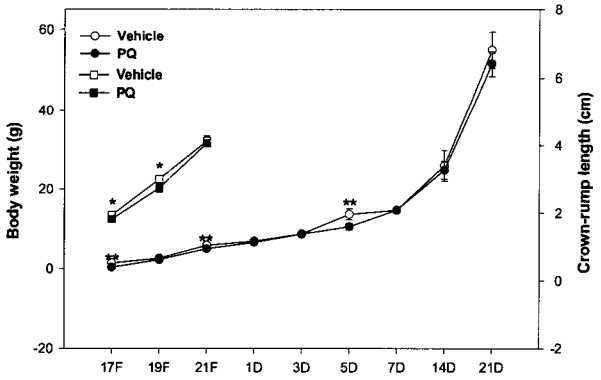

Fig. 1. Measurements of body weights and crown-rump length during rat development (mean±SD). F, fetal rat; D, days old rat; crown-rump length (□); body weight (○). * $P < 0.05$, ** $P < 0.01$. n = 8.

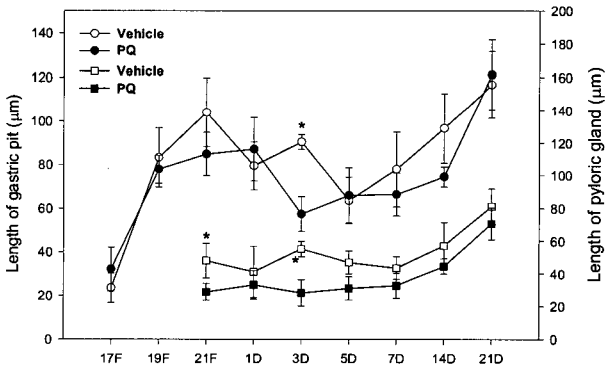

Fig. 2. Lengths of pyloric glands (□) and their pit (○) during rat development (mean±SD). * $P < 0.05$. Abbreviations are given in Fig. 1. n = 8.

Table 2. GCs properties of pyloric region of the stomach by conventional methods

Stains	Region	Fetal rat			Days old rat						
		17	19	21	1	3	5	7	14	21	105
PAS	SCC	0-2/0	2-3/0-1	2-3	3-4/2-3	3-4	3-4	3-4	3-4	3-4	4
	GPCC	0-2/0	2-3/0-1	2-3/1-2	3-4/2-3	3-4	3-4	3-4	3-4	3-4	4
	UPG			0-2	2-3	2-3	2-3	2-3	2-3	2-3	1-2
	LPG					2-3	2-3	2-3	2	1-2	1
AB at pH 2.5	SCC	0	0	0	0	0	0	0	0	0	0
	GPCC^{+}	0-1/0	0-1	0	0	0	0-1	0-2	1-2/0-2	1-2	1-2
	UPG			0-1	0-1	0-1	1-2/0-1	1-2/0-1	1-2/0-2	1-2	1-2
	LPG					1-2/0-1	2/0-1	2-3/1-2	2-3/0-2	2-3	3
AB at pH 1.0	SCC	0	0	0	0	0	0	0	0	0	0
	GPCC^{+}	0	0	0	0	0	0-2	0-2	0-2	2-3/1-2	1-2
	UPG			0	0	0	0	0	0-1	0-1	3
	LPG					0	0	0	0	0-2/0-1	3
AF at pH1.7-AB at pH 2.5	SCC	0	0	0	0	0	0	0	0	0	0
	GPCC^{+}	0-1B/0	0-1B	0	0	0	0	0-2P	2P	3P/2P	0-1P
	UPG			0-1B	0-1BP/0-1B	0-1BP/0-1B	1BP/0-1B	1BP/1B,1BP	1BP	1BP,1P	2-3P
	LPG					0-1BP/0-1B	1BP/0-2B	2BP/1-2B	2BP/2B	2BP,2P/2BP,2B	2P

The Numbers indicated relative intensity of the reaction: 4, very intense; 3, intense; 2, moderate; 1, weak; 0, absent; SCC, surface columnar cell in the area of pyloric gland; GPCC, gastric pit columnar cells in the area of pyloric gland; UPG, upper part of the pyloric gland; LPG, lower part of the pyloric gland; PAS, periodic acid Schiff's reaction, AB, alcian blue, AF, aldehyde fuchsin; P, purple; B, blue; BP, bluish purple; /, vehicle/PQ-treated group showed a different staining; $^{+}$, acidic GCs producing cells are located at the bottom in the pyloric pit.

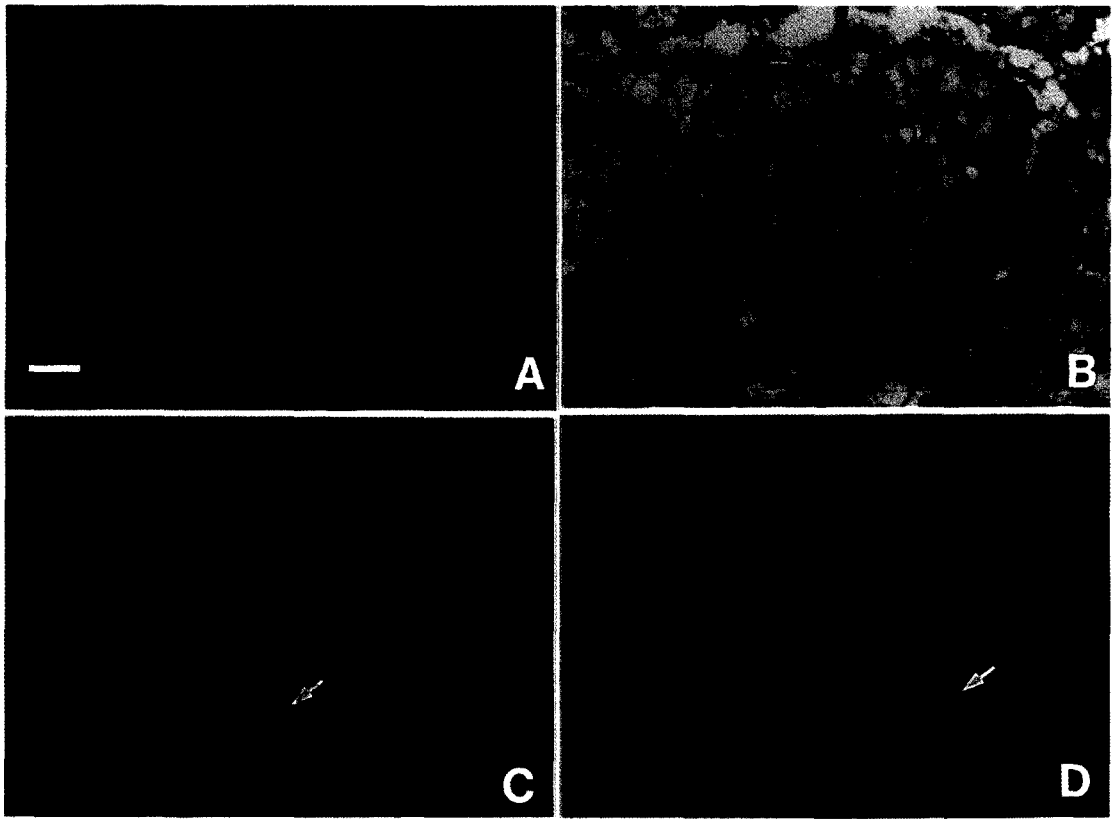

Fig. 3. Conventional GCs histochemistry for the pyloric region of the stomach. Intensive PAS reactions were detected both in the vehicle (A) and PQ-treated 3 days old rat (B) on the surface and pit columnar cells. The length of pyloric glands and their pit of the PQ-treated rat showed growth retardation. As for properties of acidic GCs determined by a combination staining of AF at pH 1.7 and AB at pH 2.5, bluish purple (a mixture of sulfated and nonsulfated GC) and blue (nonsulfated GCs) staining were observed in the pyloric gland (arrows) of 14-day old rats of vehicle (C) and PQ-treated group (D), respectively. Scale bar = 30 μm.

Table 3. GCs properties of pyloric region of the stomach by lectin histochemistry

Stains	Region	Fetal rat			Days old rat						
		17	19	21	1	3	5	7	14	21	105
DBA	SCC	0	0-2	2-3/1-2	1-2	1-2	1-2	1-2	2	2	2-3
	GPCC	0	0-2	2-3/1-2	1-2	1-2	1-2	1-2	2	2	2-3
	UPG			1-2/0-2	2	1-2	1-2	1-2	3	3-4	3-4
	LPG					2-3/1-2	2-3/1-2	2-3	0-3/3	0	0
SBA	SCC	0-2/0	1-2	2-3/1-2	2-3/1	1-2	1-2	1-2	1-2	1-2	1-2
	GPCC	0-2/0	2-3	2-3/1-2	2-3/1	1-2	1-2	1-2	1-2	1-2	1-2
	UPG			3/0-1	2/0-1	1-2/0-1	1-2/0-2	1-2	2-3	2-3	2-3
	LPG					3/0-1	2/0-2	2	0-3/2-3	0	0
PNA	SCC	0-2/0-1	2	2/0	0-1/0	1/0	1/0-1	1	1	1	1
	GPCC	0-2	3	1-2/0-1	0-1	1/0-1	1	1	1	1	1
	UPG			2/0-2	2/0-2	2/0-2	0-2	0-1	1-2	1-2	0-2
	LPG					3/0-2	0-2	0-2	0-2	0/0-1	0
BSL-1	SCC	0	0-2/0-1	1	1	1	1-2	1-2	1-2	1-2	1-2
	GPCC	0	0-3	1	1	1	1-2	1-2	1-2	1-2	1-2
	UPG			1-2	2/1-2	2-3/1-2	2-3/1-2	2-3/1-2	2-3/1-2	2-3	2-3
	LPG					3/2	2	2	0-3/2	0/0-2	0
RCA-1	SCC	0-2	2/1	1	1	1	1	1	1	1	1
	GPCC	0-2	2-3/1-2	1	1	1	1	1	1	1	1
	UPG			1	1-2/0-1	2/0-1	1-2/0-1	1-2/0-1	1-2	1-2	1-2
	LPG					2/0-1	2/0-1	2/1	2	2	2-3
sWGA	SCC	0-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
	GPCC	0-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
	UPG			3	2-3/1-2	2-3/1	1-2	1-2	2-3	2-3	2-3
	LPG					3/1-2	2	2	3	3	3-4
UEA-1	SCC	0	0-1/0	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
	GPCC	0	0-3	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
	UPG			0-2	0-2	0-2	0-2	0-2	0/0-2	0/0-2	0
	LPG					2-3/0-1	2-3/0-2	2-3	3	3	3-4
Con A	SCC	0-1/0	0-1/0	1/0	1	1	1	1	1	1	1
	GPCC	0-1/0	0-1/0	0-1/0	1	1	1	1	1	1	1
	UPG			0	0	0	0	0-1/0	0-1/0	0-1/0	1-2
	LPG					0	0	0-1/0	0-1	0-1	1-2
LCA	SCC	0	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
	GPCC	0	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
	UPG			0	0	0	0	0	0	0	0
	LPG					0	0	0	0	0	0

Abbreviations are given in Table 2.

are outlined in Table 2. The surface epithelium cells contained neutral GCs only from fetal to adult stages. The neutral GCs predominated over the gastric pit epithelium from fetal, but acidic GCs were detected on restricted pit epithelium. Only one or two cells containing acidic GCs were first detected at the bottom of the gastric pit in the 21-day fetal rats, and sulfated GCs appeared again after birth in the lower part of the gastric pit. In the pyloric gland, the cells which contained the mixture of neutral and acidic GCs were observed from fetal to adult rats. As for the properties of acidic GCs, the mixture of nonsulfated and sulfated GCs were demonstrated, which turned to sulfated GCs in the adult rat. The properties of GCs of the PQ-treated rat showed a similar pattern to those of the normal development. However, significant difference, such as delayed initial appearance of sulfated GCs in the developing rat, was revealed (Figs. 3A-D).

Lectin histochemistry

Results using lectin histochemistry are outlined in Table 3. Although affinities for all lectins used in this study were shown in the surface epithelium, somewhat denser ones in the surface and pit epithelium involving DBA, SBA, PNA and RCA-1 were observed in the fetal rat. All affinities in these regions showed the consistent affinity after birth, but different affinities were observed in the pyloric gland according to development. DBA affinity with SBA, PNA and BSL-1 was demonstrated in the pyloric gland from fetal rats, but these affinities were not observed in the lower part of the pyloric gland from 14-day old rats. In contrast, more intensive RCA-1, sWGA and UEA-1 affinities were demonstrated in the lower part of the pyloric gland in 14-day old rats. Especially, UEA-1 affinity was not observed in the upper part of the pyloric gland and weak Con A affinity were shown in 7-day old rats.

In the fetuses and young of PQ-treated pregnant rats,

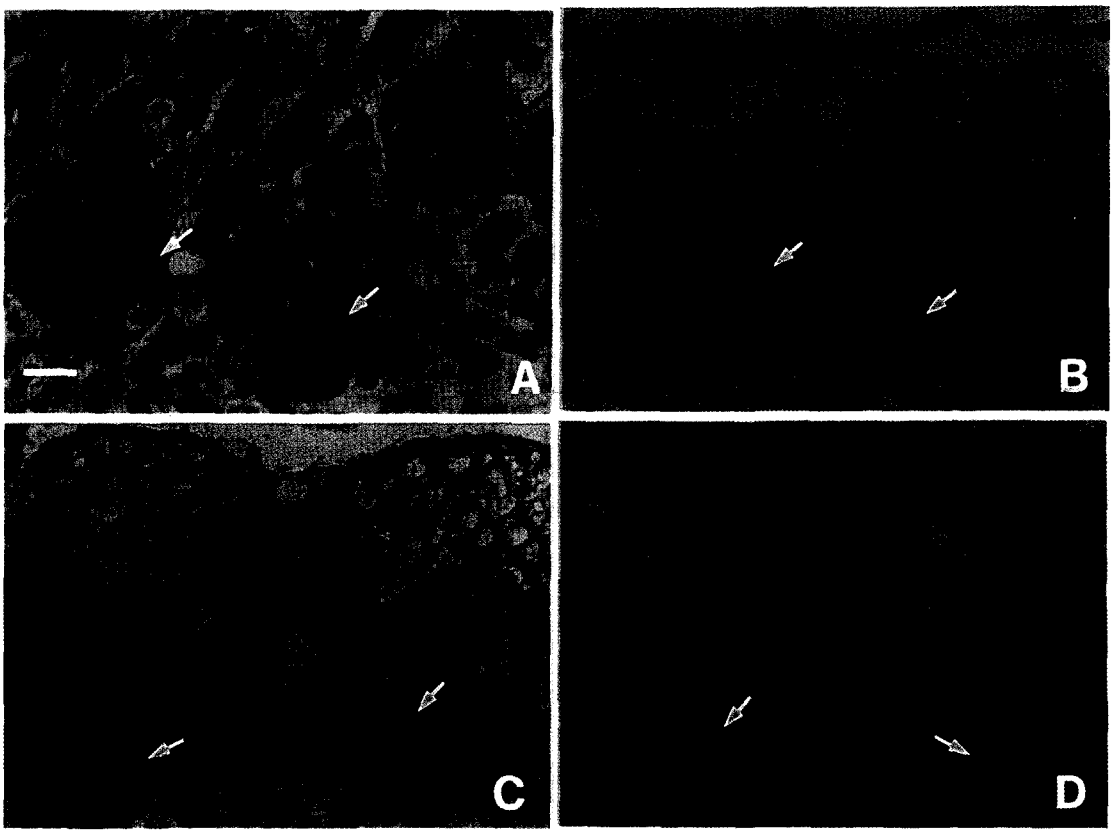

Fig. 4. Lectin histochmistry for GCs in the pyloric region of the stomach. Although the surface and pit columnar cells of the PQ-treated rat showed a similar pattern as the normal, more intensive DBA (A, B) and UEA affinities (C, D) were observed in the pyloric glands (arrows) of 3 day old rat (A, C) compared with the PQ-treated (B, D). Scale bar = 30 μm.

different lectin affinities except for LCA and sWGA were observed in both columnar and gland epithelium. These different affinities in the surface and gastric pit epithelium were mainly detected in the 21-day fetal rats. But more prominent, long-lasting and different affinities of PQ-treated rat, weaker than the vehicle group, showed in the gland epithelium after birth (Figs. 4A-D). Consequently, delayed normal GCs distribution patterns were observed in the PQ-treated rats.

Discussion

The use of herbicides for crops production has been increased. PQ as a bipyridyl compound is widely used as a contact herbicide and has been considered safe by the industry after its introduction in 1962. But the improper use of PQ has shown a significant risk in human health and the natural environment. Little is known about the relationship between its exposure to the pregnant animal and fetus.

Although the radiolabelled PQ is found in the placenta and the fetus 0.5 h after the intravenous administration to pregnant rats, PQ is considered not a teratogen (Bus et al., 1975; Selypes et al., 1980; Ingebrigtsen et al., 1984). But the drinking water supplies contaminated with herbicides elevate rates of intrauterine growth retardation (IUDR) (Munger et al., 1997) and is associated with significant reductions in gastrointestinal and pancreatic tissue weights (Xu et al., 1994).

GCs once secreted from the mucous cell in the digestive tracts play an important role as a protective barrier for the underlying epithelium (Filipe and Branfoot, 1974). They protect the epithelium from various chemicals, toxins, mechanical stimulation and various kinds of digestive enzymes (Kemper and Specian, 1991). Epithelial GCs have been classified into neutral and acidic types, with the latter subdivided into sulfated and nonsulfated groups (Sheahan and Jervis, 1976).

Generally, neutral GCs appear to be the predominant type seen in the entire gastric surface and gastric pit epithelium, with lesser amounts in the pyloric gland. Although understanding of the function of the acidic GCs still remains poor, the nonsulfated surface GCs appear to play an important role in cellular recognition, and the sulfated GCs may be related to cellular proliferation (Sheahan et al., 1970; Sheahan and Jervis, 1976).

Lectins, proteins and glycoproteins that bind with an antibody-like affinity to specific oligosaccharides in specific linkage, are useful histochemical tools for

obtaining more specific information about chemical structure of GCs. Lectin histochemistry has shown that cellular and regional differences in its affinities to gastrointestinal tract appear to reflect epithelial cell differentiation as well as regional differences in cellular carbohydrate components (Suganuma, 1985; Ihida et al., 1988).

The pre- and postnatal developmental patterns of GCs have been studied with regard to the level of enzyme activity and age-related differences in the types of GCs synthesized in the gastrointestinal tract (Kantani-Matsumoto and Kataoka, 1989). In the present study, the developmental modification in the pyloric region of the stomach are due to age-related differences in the types of GCs synthesized by rat stomach.

Our present data demonstrated that administration of PQ to pregnant rat revealed IUDR with retarded histogenesis and GCs maturation of pyloric region of the stomach. Especially, the difference in GCs properties such as the first appearance of sulfated ones related to cellular proliferation and delayed lectin affinities was shown in the surface and gastric pit epithelium of fetuses and pyloric gland of newborn rat compared with the vehicle group.

Although no data concerning the possible influence of PQ to GCs properties are available, these findings agree with earlier observations that the cells of the gastrointestinal tract in the animals with IUGR appear relatively immature. The different lengths of the pyloric gland and its pit between the PQ-treated and vehicle groups may be due to lowered cell numbers and are proportionate to the body as a whole. Our findings emphasize that PQ may be considered as a highly IUDR inducing agent in mammals and should be regulated against improper use and overuse procedures.

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