

HEP 00483

## Effect of cimetidine, ranitidine, famotidine and omeprazole on hepatocyte proliferation in vitro

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### Summary

Recently reports have indicated that both cimetidine and ranitidine delay cell proliferation in rats following 70% partial hepatectomy and result in an increased mortality following this procedure. The present study was designed to determine whether three H<sub>2</sub> blocking agents (cimetidine, ranitidine, famotidine) and a new, powerful antisecretory drug (omeprazole) specifically influence hepatocyte proliferation in primary culture. Hepatocytes were isolated from livers of normal male rats by the standard collagenase perfusion technique. Hepatic DNA synthesis and percent of labelled nuclei were determined after 48 h incubation. Hepatocytes in culture were incubated with the H<sub>2</sub> blocking agents and omeprazole or with different concentrations of serum obtained from sham-operated or 70% hepatectomized rats treated or not with the same agents. Rats were injected intraperitoneally at 8:00 a.m. on two consecutive days. In hepatectomized rats, the first dose was injected at 8:00 a.m. immediately after surgery, the second, 24 h later. The serum of sham-operated or 70% hepatectomized rats that did not receive drugs served as control. No changes in DNA synthesis, percentage of labelled nuclei and transaminase were detected when the agents were added to the hepatocytes in culture at concentrations within the effective pharmacological dosage and 30 times higher. Similarly, no changes in these parameters were obtained when different concentrations of serum obtained from sham-operated rats treated with H<sub>2</sub> blocking agents or omeprazole were added to the basal culture medium. However, a significant inhibition of DNA synthesis and of percentage of labelled nuclei was observed when hepatocytes were incubated in the presence of serum from 70% hepatectomized rats that had been treated with cimetidine or with ranitidine. The serum of 70% hepatectomized rats treated with famotidine and omeprazole had no effect on hepatocyte proliferation in vitro. No effect on transaminase was found in these conditions.

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## Introduction

It has recently been reported that the administration of cimetidine and ranitidine to rats following 70% partial hepatectomy (PH) significantly delays and inhibits hepatic DNA synthesis and the percentage of mitosis [1,2]. No direct evidence has been presented to explain the mechanism of this inhibition. Both these drugs continue to be widely used after major hepatic resection for the prophylaxis of gastric complications ('stress ulcer syndrome') [3-6] and are also widely prescribed to patients with chronic hepatic disorders and esophageal varices [7-9]. Thus, a more specific evaluation of the effect of these drugs on liver regeneration is warranted.

The experiments reported here were designed to determine the effects of these compounds on hepatocyte proliferation *in vitro*. The two widely used agents, cimetidine and ranitidine, as well as a new, potent H<sub>2</sub> blocking agent, famotidine [10], and a new inhibitor of acid secretion, omeprazole [11], were evaluated. Either the antisecretory agents or the serum taken from rats undergoing PH or sham operation and treated with these agents were tested. The H<sub>2</sub> blocking agents and omeprazole were added directly to hepatocyte cultures in serum-free medium in the presence or absence of epidermal growth factor (EGF).

## Materials and Methods

### Animals

Male Fischer F(344) rats, weighing 180-200 g, were obtained from Hilltop Lab Animals, Inc., Scottsdale, PA. The animals were housed in a temperature (20 ± 1 °C) and light (6 a.m.-6 p.m.) controlled room and received food and water *ad libitum*. Seventy percent PH was performed according to the method described by Higgins and Anderson [12]. Sham operation consisted of a laparotomy and gentle manual manipulation of the liver. All surgical procedures were performed between 7:30 and 9:00 a.m.

### Materials

Collagenase type I (125-250 U/mg) was purchased from Worthington Diagnostic Systems, Freehold, NJ. MEM (minimal essential medium) and fetal calf serum (FCS) were purchased from GIBCO Laboratories, Grand Island, NY. Insulin, EGF, HEPES, pyruvic acid and proline were purchased from Sigma Chemical Company, St. Louis, MO. [*methyl*-<sup>3</sup>H]-Thymidine (50-80 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Cimetidine was obtained from Smith, Kline and French Lab Co., Caroline, PR, ranitidine from Glaxo, Inc., Research Triangle Park, NC, famotidine from Sigma Tau Co., Rome, Italy, omeprazole from Bracco, Milan, Italy. Aqueous Counting Scintillant (ACS) was purchased from Amersham, Arlington Heights, IL.

### Isolation of hepatocytes

Hepatocytes were isolated from 7-week-old male rats weighing 180-200 g, by a modification of the *in situ* two-step collagenase perfusion technique of Seglen [13] described by Jirtle et al. [14]. The liver was perfused via a cannula in the inferior vena cava with 250 ml buffer (142 mM NaCl, 6.7 mM KCl, 10 mM HEPES, pH 7.4) followed by 250 ml of the same buffer containing in addition 5.7 mM CaCl<sub>2</sub> and 0.5 mg/ml collagenase. The hepatocytes were dispersed and washed twice with cold Ca<sup>2+</sup>-free perfusion buffer and resuspended in MEM supplemented with proline (26 mM), pyruvate (1 mM), aspartate (0.2 mM), serine (0.2 mM), gentamycin (40 µg/ml). For attachment, insulin (10<sup>-7</sup> M) and FCS (5%) were added. Viability was determined by trypan blue exclusion [15] and only preparations having greater than 90% viability were used. Cell number was determined with a hemocytometer.

The cells were plated at a cell density of 2 × 10<sup>5</sup> in 35-mm Falcon 'Primaria' tissue culture dishes in 1.5 ml medium and maintained at 37 °C and at 5% CO<sub>2</sub> atmosphere. Following a 3-h attachment period, MEM was added with the specified additions as reported. EGF and insulin, when added, were at a concentration of 10 ng/ml and 10<sup>-7</sup> M, respectively.

#### *Determination of [<sup>3</sup>H]thymidine incorporation and DNA synthesis*

After a 3-h period, necessary for the hepatocyte attachment, the medium was changed and specific additions were made. [<sup>3</sup>H]Thymidine, 7.5  $\mu$ Ci/dish, was present from 24–48 h or from 48–72 h. Each experimental group comprised four dishes. When the cells were harvested, two dishes were used to determine DNA content using the microfluorometric method of Setaro and Morley [16], and two dishes of each group were treated as described by Michalopoulos et al. [17] to measure [<sup>3</sup>H]thymidine incorporation.

#### *Serum studies*

Forty normal rats were sham-operated, divided into five groups and injected as follows: group I, cimetidine (2.4 mg/200 g body wt.); group II, ranitidine (0.8 mg/200 g body wt.); group III, famotidine (0.11 mg/200 g body wt.); group IV, omeprazole (0.06 mg/200 g body wt.); group V, saline. Another 40 rats underwent PH, were divided into five groups and injected in the same manner as the sham group. All animals were operated on before 8 a.m. and all were injected i.p. at 8 a.m. on the day of operation and at 8 a.m. the following day with the drugs dissolved in saline (0.5 ml) with the exception of omeprazole which was dissolved in PEG-500. Under ether anesthesia, blood was taken from the animals 3 h after the final injection. Blood samples of these animals, as well as blood taken from a group of eight normal unhepatectomized rats, were taken for hormonal study.

#### *Autoradiography*

For autoradiographic studies, hepatocytes plated on 'Primaria' dishes were fixed and stained for glucose-6-phosphate dehydrogenase directly on the dishes, as reported by us [18]. [<sup>3</sup>H]Thymidine, 7.5  $\mu$ Ci/dish, was present for 24 h prior to fixation. The dishes were covered with emulsion (Kodak NTB3) and developed after 10 days. The labelling index was determined by counting a total of 1000 cells per dish. The glucose-6-phosphate dehydrogenase positivity clearly showed that the cultured cells were parenchymal hepatic cells [18].

#### *Hormone studies*

The plasma levels of immunoreactive insulin (IRI) were determined by radioimmunoassay using an insulin kit obtained from Serono Diagnostics (Brain-tree, MO). The detection limit of the assay was 5  $\mu$ U/ml.

The plasma levels of immunoreactive glucagon (IRG) were determined with a glucagon kit obtained from Serono Diagnostics. This kit was specifically chosen for its high degree of accuracy, precision and specificity as has been reported [19,20]. Samples for the glucagon assay were collected in chilled tubes containing 500 units of a trypsin inhibitor (aprotinin) and 1.2 mg of sodium EDTA/ml of whole blood collected for assay. The detection limit for the assay was 15 pg/ml. The results were also expressed as insulin/glucagon molar ratio which was calculated as suggested by Muller [21] using the formula  $[\text{IRI} (\mu\text{U/ml})/\text{IRG} (\text{pg/ml})] \times 23.33 = \text{ratio}$ . This formula assumes that insulin has a molecular weight of 6000 Da and a biologic activity of 25 units/mg. It also assumes that glucagon has a molecular weight of 3500 Da. Serum testosterone and estradiol were determined by specific radioimmunoassays as described previously [22].

#### *Cimetidine determination*

The blood level of cimetidine was determined with HPLC using the method of Mihaley et al. [23]. Blood was taken under ether anesthesia from three untreated sham and PH rats (control), nine sham-operated and nine PH rats treated with cimetidine as reported in Materials and Methods. In the untreated rats, the blood was taken at 8 a.m. and used as a control. In the sham-operated and the PH operated rats (three from each group), blood was taken 1, 2 and 3 h after the last injection of cimetidine on the second day of treatment.

#### *Determination of aminotransferases in the medium*

Hepatocytes were seeded as described above, and test compounds were added 3 h later (first medium change) at the concentrations indicated in Table 1. The medium, after 48 h of incubation, was centrifuged at 2000  $\times$  g for 15 min, and an aliquot of the su-

pernatant was diluted with an equal volume of phosphate-buffered saline. Activity of alanine aminotransferase (ALT) (EC 2.6.1.2) was determined with a spectrophotometer (Perkin-Elmer Coleman 124D, Maywood, IL) by the lactate dehydrogenase coupling method as originally described by Karmen [24,25]. The activities of transaminase in the medium are expressed in U/ml. One unit of activity is defined as the amount of enzyme, which produces 1  $\mu$ mol of NAD per min under the conditions of the assay procedure.

#### Statistical study

The unpaired Student's *t*-test was used for statistical analysis of the data using the program available on the Hewlett-Packard 9815S (Hewlett-Packard Co., Palo Alto, CA).

### Results

Table 1 demonstrates the results of cimetidine, ranitidine, famotidine, omeprazole and saline treatment on the viability, DNA, and ALT of hepatocytes in primary cultures. As is reported in our previous paper [18], 98% of cells attached to the dish were

hepatocytes. The H<sub>2</sub> blocking agents were added to hepatocytes cultured in serum-free medium in the presence of insulin alone ( $10^{-7}$  M). This medium is optimal for preserving all the metabolic properties of hepatocytes without promoting proliferation. Moreover, it is ideal for metabolic as well as toxicological studies. The drug concentrations listed in Table 1 are 30 times higher than the maximum concentration found in the blood of patients and animals treated with a single therapeutic dose of H<sub>2</sub> blockers [26–29]. There were no significant differences in the viability or DNA concentration of hepatocytes and transaminase in the medium in the presence or absence of the H<sub>2</sub> blocking agents and omeprazole, suggesting that these drugs have no direct toxic effect on hepatocytes in culture.

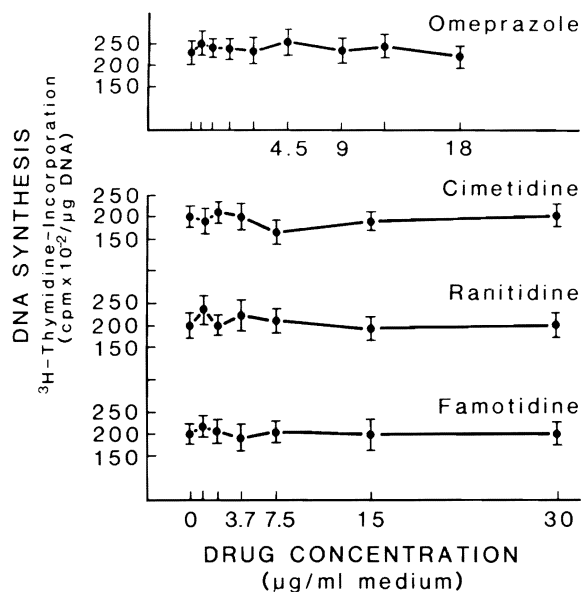
Fig. 1 depicts the effect of various concentrations of cimetidine, ranitidine, famotidine and omeprazole on the proliferation of hepatocytes cultured for 48 h in serum-free medium. Conditions were the same as in the previous experiment, with the exception of an addition of EGF to the medium which promoted hepatocyte proliferation. None of these drugs affected the hepatocyte proliferation stimulated by EGF. Further confirmation of these results is reported in Table 2, which lists the concentration of DNA and the per-

TABLE 1

EFFECT OF CIMETIDINE, RANITIDINE, FAMOTIDINE AND OMEPRAZOLE ON VIABILITY, DNA CONCENTRATION AND TRANSAMINASE LEVELS OF HEPATOCYTES IN 'PRIMARY CULTURES'

Hepatocyte isolation has been described under 'Materials and Methods'. Cells were plated in MEM supplemented with 5% FCS and insulin ( $10^{-7}$  M). After 3 h attachment, the medium and floating cells were removed and serum-free MEM plus insulin ( $10^{-7}$  M) was added. Antisecretory agents were added as indicated. Cells were cultured for 72 h and processed every 24 h for viability and DNA determinations. ALT was determined and expressed as U/ml only for the dishes stopped after 72 h of incubation. For each time point, triplicate culture dishes were assayed. The results are expressed as the average from 9 dishes and 3 different animals (mean  $\pm$  S.D.).

	24 h		48 h		72 h		ALT (U/ml)
	Viability (%)	DNA ( $\mu$ g/dish)	Viability (%)	DNA ( $\mu$ g/dish)	Viability (%)	DNA ( $\mu$ g/dish)	
Control	95 $\pm$ 3	3.2 $\pm$ 0.2	97 $\pm$ 3	3.3 $\pm$ 0.2	98 $\pm$ 2	3.28 $\pm$ 0.3	9 $\pm$ 3
Cimetidine 27 $\times$ 10 <sup>-6</sup> g/ml	97 $\pm$ 2	3.1 $\pm$ 0.2	98 $\pm$ 2	3.2 $\pm$ 0.3	98 $\pm$ 1	3.28 $\pm$ 0.2	11 $\pm$ 5
Ranitidine 6.0 $\times$ 10 <sup>-6</sup> g/ml	98 $\pm$ 1	3.1 $\pm$ 0.3	97 $\pm$ 3	3.4 $\pm$ 0.35	96 $\pm$ 4	3.05 $\pm$ 0.4	10 $\pm$ 9
Famotidine 2.1 $\times$ 10 <sup>-6</sup> g/ml	95 $\pm$ 2	3.2 $\pm$ 0.1	96 $\pm$ 1	2.95 $\pm$ 1.2	97 $\pm$ 1	3.08 $\pm$ 0.7	12 $\pm$ 3
Omeprazole 1.8 $\times$ 10 <sup>-6</sup> g/ml	95 $\pm$ 1	3.2 $\pm$ 0.1	97 $\pm$ 2	2.9 $\pm$ 1.1	97 $\pm$ 2	3.3 $\pm$ 0.9	8 $\pm$ 2



**Fig. 1.** Effect of cimetidine, ranitidine, famotidine and omeprazole on DNA synthesis of hepatocytes in primary culture. Hepatocyte isolation has been described under 'Materials and Methods'. Cells were plated in MEM supplemented with 5% FCS and insulin ( $10^{-7}$  M). After 3 h, the medium and floating cells were discarded and serum-free medium plus insulin ( $10^{-7}$  M), EGF (10 ng/ml) and proline (0.26 mM) were added.  $H_2$  blocking agents were added at different concentrations on the basis of their blood level after administration of a therapeutic dose: cimetidine, ranitidine, famotidine (0.62–30  $\mu$ g/ml) and omeprazole (0.06–1.8  $\mu$ g/ml). Cultures were exposed to 7.5  $\mu$ Ci [ $^3$ H]thymidine for the last 24 h and processed after 48 h for determination of DNA synthesis. For each medium, triplicate culture dishes from 5 different animals were used. Results (mean  $\pm$  S.D.) are expressed as  $\text{cpm} \times 10^{-2}/\mu\text{g DNA}$ .

cent labelled nuclei index of hepatocyte proliferation. This stimulation of hepatocyte proliferation over that observed in the culture incubated in the presence of insulin alone was not affected by the presence of drugs. The same results were obtained when, under the same conditions, serum of PH or non-PH rats was added. Fig. 2 outlines the effect of various serum concentrations (10–50% final concentration) on the DNA synthesis of hepatocytes in culture. Serum was obtained from sham-operated animals which had received i.p. cimetidine, ranitidine, famotidine, omeprazole and saline. No statistical difference was found in DNA synthesis between any of these groups. The effect of serum, obtained from rats

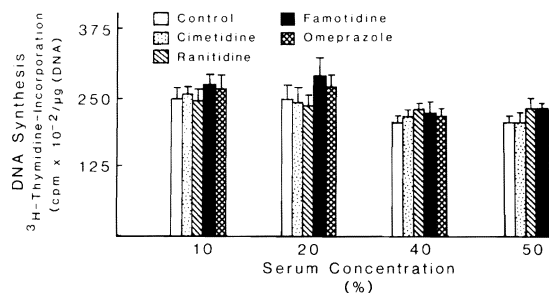
TABLE 2

DNA CONCENTRATION AND LABELLING INDEX OF HEPATOCYTE CULTURES GROWN IN THE PRESENCE OF CIMETIDINE, RANITIDINE, FAMOTIDINE, AND OMEPRAZOLE

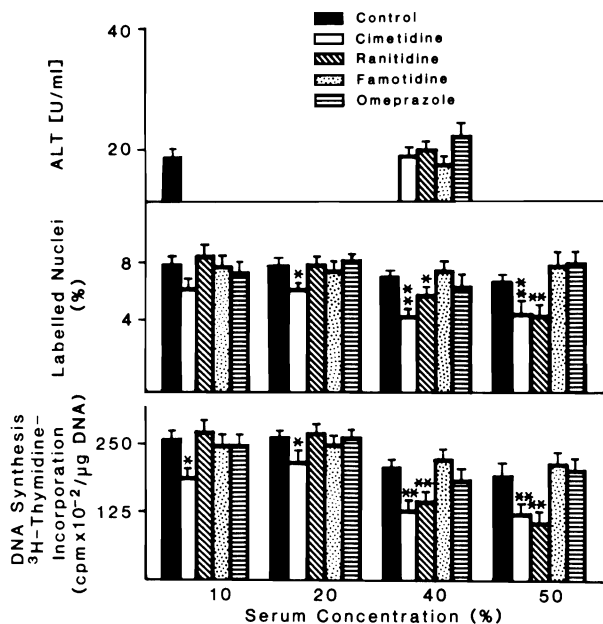
Culture conditions were as for Fig. 1. After 48 h of incubation, dishes were processed for DNA concentration and % labelled nuclei. Concentrations of  $H_2$  blocking agents were 30 times higher than the blood value found in patients injected with one pharmacological dose of the drugs. Cultures without  $H_2$  blocking agents were used as control. DNA concentration was expressed as  $\mu\text{g}/\text{dish}$ . The results are expressed as the average from 9 dishes and 3 different animals (mean  $\pm$  S.D.). DNA in the labelled nuclei of hepatocytes incubated in the presence of insulin alone was  $2.8 \pm 0.33 \mu\text{g}/\text{dish}$ ; percent of labelled nuclei in the same conditions was  $4.5 \pm 7$ .

	DNA ( $\mu\text{g}/\text{dish}$ )	Labelled nuclei (%)
Control (saline)	$6.2 \pm 1.2$	$10 \pm 1$
Cimetidine $27 \times 10^{-6}$ g/ml	$5.2 \pm 1.8$	$9 \pm 2$
Ranitidine $6.0 \times 10^{-6}$ g/ml	$5.8 \pm 1.2$	$10 \pm 2$
Famotidine $2.1 \times 10^{-6}$ g/ml	$5.5 \pm 2.0$	$12 \pm 2$
Omeprazole $1.8 \times 10^{-6}$ g/ml	$5.9 \pm 1.8$	$11 \pm 3$

that underwent PH and received  $H_2$  blocking agents and omeprazole, on DNA synthesis and percentage of labelled nuclei is depicted in Fig. 3. The DNA syn-



**Fig. 2.** Effect of serum obtained from rats sham-operated and treated with  $H_2$  blocking agents and omeprazole on DNA synthesis of hepatocytes in primary culture. The experimental conditions are described in 'Materials and Methods'. Culture conditions were as in Fig. 1, with the exception that serum from rats treated with or without (as control)  $H_2$  blocking agents were added instead of EGF. Four concentrations (10–50%) of serum were used. Results (mean  $\pm$  S.D.) are expressed as  $\text{cpm} \times 10^{-2}/\mu\text{g DNA}$ . For each medium, triplicate culture dishes from 5 different experiments were used.



**Fig. 3.** Effect of different concentrations of serum obtained from 70% hepatectomized rats treated or not with cimetidine, ranitidine, famotidine and omeprazole on DNA synthesis, % labelling index and ALT of hepatocytes in primary culture. The experimental conditions are described in 'Materials and Methods'. Culture conditions were as reported in Fig. 1, with the exception that serum from treated and not treated (as control) 70% hepatectomized rats was added instead of EGF. Four final concentrations (10, 20, 40 and 50%) of serum were used. Exposure to [<sup>3</sup>H]thymidine was for 24 h. Individual culture dishes were processed for autoradiography and DNA synthesis. A total of 1000 cells randomly selected were counted to obtain the percent of labelled cells. Results are presented as mean ± S.D. (DNA synthesis = cpm × 10<sup>-2</sup>/μg DNA; labelled nuclei = % of labelled nuclei; aminotransferase = U/ml). For each medium, triplicate culture dishes from 5 different experiments were used. Significantly different from the control value observed in each group: \**P* < 0.05; \*\**P* < 0.01.

thetic activity of hepatocytes cultured in the presence of different concentrations of serum from hepatectomized rats treated with the drugs was compared with the values obtained using the serum from hepatectomized rats treated with saline. The serum of PH rats treated with cimetidine inhibited DNA synthesis and percent of labelled nuclei at all the concentrations of serum used. Although the inhibitory effect of cimetidine was partially covered by the different serum concentrations, the percent of the effect increased in relation to the amount of serum used (10% serum =

29% inhibition; 40% serum = 38% inhibition). The serum of PH rats injected with ranitidine inhibited hepatocyte proliferation only at the higher serum concentrations, i.e., 40–50%. No inhibitory effects were observed using the serum of PH animals treated with famotidine or omeprazole. The figure also shows the value of ALT in these conditions. No significant variation of ALT was observed in the presence of 40% serum concentrations.

Table 3 reports the values of glucagon, insulin, insulin/glucagon ratio, estrogen and testosterone in sham-operated rats and in rats who underwent a 70% PH 24 h before, treated and not treated with antisecretory substances. The significant variation due to the surgical procedures (*P* < 0.05) for insulin, glucagon and testosterone was not affected by the administration of H<sub>2</sub> blocking agents or omeprazole. The hormonal determinations were done in five animals of each group.

Fig. 4 reports the level of cimetidine in sham-operated and PH rats treated with cimetidine as is reported in Materials and Methods. Cimetidine was not found in the blood of untreated sham and PH rats. Sham-operated treated rats have high blood levels of cimetidine 1 h after its administration, which decreases over a period of 3 h. In contrast, the blood level of cimetidine in PH-treated rats remained constant over the same period. We only studied the levels of cimetidine in these experiments since it demonstrated the most significant effect on regeneration. We did not measure cimetidine level immediately after surgery to avoid any influence of surgical stress and/or anesthesia in drug kinetics.

## Discussion

Two commonly used H<sub>2</sub> blocking agents, cimetidine and ranitidine, have been demonstrated recently to interfere significantly with DNA synthesis in a model of 70% partially hepatectomized rats [1,2]. Although the change (hemodynamics) produced by these agents was ruled out as a possible mechanism of action [2], no further attempts to define the basis of this observation have been reported. It is now well

TABLE 3

DETERMINATION OF INSULIN, GLUCAGON, IRI/IRG, ESTROGEN AND TESTOSTERONE IN SHAM-OPERATED RATS AND IN PH RATS TREATED IN OUR STUDY WITH H<sub>2</sub> BLOCKING AGENTS OR OMEPRAZOLE

	Glucagon (pg/ml)	Insulin ( $\mu$ U/ml)	IRI/IRG	Estrogen (pg/ml)	Testosterone (ng/ml)
Sham rats					
control	180 $\pm$ 56	27 $\pm$ 2	3.50	8.3 $\pm$ 3	1.7 $\pm$ 0.33
cimetidine	173 $\pm$ 68	24 $\pm$ 3	3.23	7.3 $\pm$ 2	1.3 $\pm$ 0.35
ranitidine	183 $\pm$ 22	18 $\pm$ 7	2.29	3.5 $\pm$ 5	1.4 $\pm$ 0.15
famotidine	198 $\pm$ 64	28 $\pm$ 2	3.29	10.3 $\pm$ 4	1.5 $\pm$ 0.40
omeprazole	193 $\pm$ 85	25 $\pm$ 3	4.10	6.4 $\pm$ 3	2.0 $\pm$ 0.50
70% PH rats					
control	410 $\pm$ 110	20 $\pm$ 2.0	1.14	7.9 $\pm$ 2.1	0.30 $\pm$ 0.03
cimetidine	460 $\pm$ 150	24 $\pm$ 2.4	1.20	7.8 $\pm$ 3.0	0.95 $\pm$ 0.01
ranitidine	540 $\pm$ 100	29 $\pm$ 3.0	1.25	6.9 $\pm$ 4.0	0.18 $\pm$ 0.02
famotidine	480 $\pm$ 90	26 $\pm$ 1.5	1.26	9.3 $\pm$ 3.0	0.32 $\pm$ 0.03
omeprazole	420 $\pm$ 70	27 $\pm$ 7.2	1.50	8.1 $\pm$ 2.0	0.48 $\pm$ 0.04

Control, saline; cimetidine, 2.4 mg/rat; ranitidine, 0.8 mg/rat; famotidine, 0.11 mg/rat; omeprazole, 0.06 mg/rat.

established that hepatocytes in culture with serum-supplemented or serum-free medium can be maintained for several days and can be induced to synthesize DNA by providing certain hormones or growth factors [15,18,30–33]. This represents a model in vitro system to evaluate candidates which are proposed to interfere with hepatocyte proliferation. This system has been used in the present studies as the basis for defining the effect of three H<sub>2</sub> blocking agents (cimetidine, ranitidine, famotidine) and a gastric secretion

inhibitory agent (omeprazole).

The results reported here clearly demonstrate that first- (cimetidine and ranitidine) and second (famotidine)-generation H<sub>2</sub> blocking agents and omeprazole do not exert any measurable direct control on hepatocyte proliferation in vitro. The addition of these agents, also when used at very high concentrations (30 times the active pharmacological dose found in the blood of patients treated with a single therapeutic dosage of the drugs), does not affect the viability, the DNA content or the proliferating activity of hepatocytes maintained in primary culture for a period of 72 h.

To further define their possible influence on hepatocyte proliferation, the effect of serum obtained from rats treated with H<sub>2</sub> blocking agents or omeprazole was studied. Two sets of experiments were compared, one using the serum of sham-operated animals, the other using serum of 70% hepatectomized rats. The most significant results were observed when the serum of 70% hepatectomized rats treated with the drugs was added to the culture medium (Fig. 3). Serum from hepatectomized rats treated with cimetidine inhibited hepatic proliferation in a manner that was somewhat related to the concentration of serum used. Serum of PH animals treated with ranitidine resulted in inhibition of DNA synthesis only at

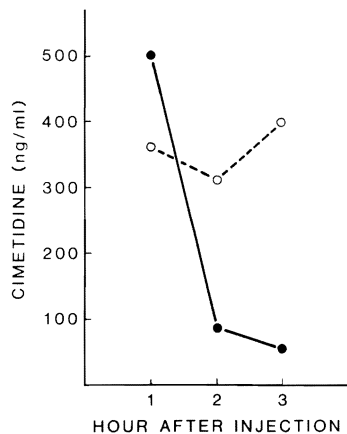


Fig. 4. Cimetidine levels in sham-operated (●—●) and PH (○--○) rats.

very high concentrations, while the serum of PH animals treated with famotidine and omeprazole did not affect hepatic proliferation at any of the concentrations used. It is evident from these studies, therefore, that the significant inhibition of DNA synthesis and percent labelled nuclei that is modulated by cimetidine and ranitidine is not due to the actual drug but is manifested by their ability to induce some variation(s) in the composition of serum during the course of hepatic regeneration.

Many recent investigations and reviews have focused on the permissive effect, or possibly the initiating role, of hormones in hepatic regeneration [34–36]. Insulin and glucagon were the first two hormones whose so-called hepatotropic action was associated with liver regeneration [37,38]. Since then, many studies have shown that other hormonal changes, especially those related to sex hormones, occur during the first 48 h of liver regeneration [39–49].

On the other hand, the ability of H<sub>2</sub> blocking agents to interfere with the hormonal state has been well known since the beginning of the clinical use of cimetidine [50]. Variations of the hypothalamic-pituitary-gonadal axis have been reported during treatment with cimetidine which have been associated with increased serum prolactin levels, impotence and gynecomastia [51,52].

Only a few reports regarding these effects are described for ranitidine [51–54] while none have been reported for famotidine or omeprazole [55]. The data reported here demonstrate that insulin and glucagon as well as estrogen and testosterone blood levels do not change in the PH animal despite the treatment regimen imposed on the rats. Therefore, the inhibitory effect on hepatic proliferation is not due to the hormonal changes measured in these studies in the se-

rum of PH rats. The influence of other hormones and growth factors (e.g., pituitary, thyroid, etc.) on the results reported here cannot be ruled out.

The only difference found by us was the higher level of cimetidine in the serum of PH rats compared with that observed in the serum of sham-operated rats. This difference suggests that either the liver has an important role in the catabolism of cimetidine or that increased blood cimetidine levels following PH are due to some degree of renal dysfunction.

Such mechanisms could be acting under the conditions described here; in fact, the effect found by us using serum from PH rats treated with cimetidine could be due to the metabolites of this substance.

These results are not in contrast to the demonstration that the H<sub>2</sub> blocking agents, added directly to the hepatocytes cultured *in vitro*, have no effect in spite of the high concentration used. It is known that when hepatocyte proliferation is studied *in vitro*, one of the optimal conditions is low cellular density in the culture in order to prevent the phenomenon of the contact inhibition of proliferation. The amount of metabolite produced by such a low cellular population could not reach an effective concentration in the medium, capable of inhibiting hepatocyte proliferation. This same phenomenon could be true for ranitidine, which has a similar inhibitory activity of hepatocyte proliferation at a much higher concentration.

Based on these results, further experiments are in progress to assess hepatocyte proliferation in culture in the presence of cimetidine and ranitidine metabolites to completely clarify this phenomenon. In the interim, we emphasize that our results, confirming those found by Kanashima *in vivo*, focus attention on the need for caution and careful evaluation when this drug is administered to the patient immediately following hepatectomy.

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