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Circadian reduction of chromium in the gastric environment

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Summary

Samples of gastric juice from variously treated subjects efficiently reduced hexavalent chromium and decreased its mutagenicity. Chromium reduction was due to thermostable components of gastric secretions and was favoured by the acidity of the intragastric environment. The circadian monitoring of pH and of chromium reduction, as assessed by colorimetric analysis at hourly Intervals, showed a basal activity (less than 10 μg/ml gastric juice) during the night and interdigestive periods, and peaks (tens of μg/ml) during the 3–4-h periods after each meal. Assays in the Ames reversion test confirmed that the decrease in mutagenicity of sodium dichromate produced by gastric juice was significantly enhanced alter meals. This physiological mechanism is expected to provide an important protective barrier against the oral tosicity of this metal, and may explain its lack of oral carcinogenicity.

Toxic, mutagenic and carcinogenic effects of chromium are strictly dependent on its oxidation state, Cr(VI) being the active form (IARC, 1980). Accordingly, chemical or metabolic oxido-reductive mechanisms occurring in the organism play a crucial role in chromium toxicology. While the stable reduced form, i.e. Cr(III), is not expected to be oxidized to active Cr(VI) in the organism (Petrilli and De Flora, 1978b), the reverse process has been demonstrated to occur in some body fluids [e.g. in plasma (Korallus et al., 1974) and in secretions of the alimentary tract (Petrilli and De Flora, 1982)], in transport cells [e.g. in erythrocytes (Gray and Sterling, 1950; Petrilli and De Flora, 1978a)], in phagocytic cells [e.g. in alveolar macrophages (Petrilli et al., 1986a)] and in several cells of various animal species, including humans (Petrilli et al., 1986b). The underlying biochemical mechanisms have been also explored (De Flora et al., 1985; Petrilli et al., 1985).

The efficiency of Cr(VI) reduction in various body fluids and cell compartments is likely to constitute a threshold mechanism limiting the in vivo activity of this metal and an important factor in the selection of target cells (Petrilli et al., 1986b). For instance, reduction in the alimentary tract pro-

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Abbreviations: Cr(III), trivalent chromium; Cr(VI), hexavalent chromium; DPC, *s*-diphenylcarbazide.

vides a primary defence mechanism against ingested chromium. So far, the Cr(VI)-reducing ability of gastric juice had been only reported with samples obtained from fasting individuals (Donaldson and Barreras, 1966; De Flora and Boido, 1980), which may result in a considerable underestimation of this phenomenon. In the present paper we report the results concerning the circadian monitoring of Cr(VI) reduction by gastric juice.

Patients and methods

Patients

The study was carried out with the informed consent and full cooperation of 1 healthy volunteer and 16 hospitalized patients. Most patients were suffering from duodenal ulcer, since they had been included in the protocol of a parallel study on the effects of famotidine (Merck and Co., Inc., Rahway, NJ) — a recently developed antagonist of histamine H_2-receptors — on various gastric juice parameters. Three ulcer patients, two of them also treated with misoprostol (G.D. Searle and Co., Skokie, IL) — a synthetic prostaglandin E_1 analog — were administered 1 g Vitamin C (Roche, Basel, Switzerland) just before lunch. Additionally, 3 fasting patients received an i.m. injection of pentagastrin (6 μg/kg b.w.) (Bracco Industria Chimica S.p.A., Milan, Italy), and samples of gastric juice were collected just before the injection and after 15, 30, 45 and 60 min. The dietary conditions were standardized for all subjects and consisted of 3 meals, i.e. breakfast at 8 a.m. (11.2 g protein, 13.2 g fat and 54.3 g carbohydrate), lunch at noon (52.3, 33.3 and 136.6 g, respectively) and dinner at 6 p.m. (30.4, 35.8 and 107.0 g, respectively), for a total daily energy intake of 9661 kJ.

Monitoring of intragastric pH and collection of gastric juice

A continuous intragastric pH monitoring, performed as described elsewhere (De Flora et al., 1987), was associated with hourly aspiration of gastric juice by means of a nasopastric tube. Immediately after collection, gastric juice samples were dividec into small aliquots and stored at −80°C until use.

Evaluation of Cr(VI) reduction

Gastric juce samples were thawed and centrifuged at 500 × *g* for 10 min. The supernatants were assayed for their ability to reduce Cr(VI), tested as socium dichromate ($Na_2Cr_2O_7 . 2H_2O$) (Merck-Schuchardt, Munich, F.R.G.). Each sample was testec in triplicate. All the samples having a comparative interest — e.g. all the hourly samples of a single patient — were assayed in the same experiment. Unless otherwise specified, a fixed amount (25 μl) of a chromium water solution (573 μg dichromate/ml, i.e. 200 μg Cr(VI)/ml) was mixed with a fixed amount of gastric juice (100 μl), in 10-ml tubes. After gentle mixing in a rotary shaker (10 rpm) at 37°C for 1 h, the tubes were transferred into an ice-cold bath. Immediately, two 50-μl alicuots of each mixture were directly transferred into 2 cuvets. One series of cuvets (samples) was then filled with 2.5 ml of *s*-diphenylcarbatide (DPC) reagent (40 mg DPC in 100 ml of 19% ethanol/8% sulfuric acid in water). The second series of cuvets (corresponding blanks) was filled with 2.5 ml of the same acid–ethanol mixture, but without DPC. After 15 min at room temperature, the resulting Cr(VI)-DPC complex was measured at 540 nm in a Hitachi U-3200 spectrophotometer Detailed calibration curves were drawn by testing standard solutions of untreated sodium dichromate.

Mutagenicity assays

The ability of gastric juice samples to decrease the direct mutagenicity of Cr(VI) was assayed in the Ames reversion test (Maron and Ames, 1983). using TA102, which is the most sensitive *his- S. typhimurium* strain in revealing Cr(VI) mutagenicity (Bennicelli et al., 1983). The assays were performed in the absence of exogenous metabolic systems, which are known to substantially lessen Cr(VI) genotoxicity (De Flora, 1978; Petrilli and De Flora. 1978a; Petrilli et al., 1985, 1986b). Aqueous solutions of sodium dichromate were mixed and preincubated either with bidistilled

water (Cr(VI) controls) or with each gastric juice sample (in triplicate tubes), as described in the previous sub-section. After preincubation, the mixtures were neutralized with a 4-fold excess (v/v) of phosphate-buffered saline, pH 7.4, and assayed according to the standard plate incorporation test. Cr(VI)-free plates were also assayed in order to assess the number of spontaneous revertants of the bacterial tester strain.

Results

Colorimetric assays showed that virtually all samples of gastric juice were capable of decreasing the Cr(VI)-DPC reaction, and that such effect was inhibited by a strong oxidant, i.e. potassium permanganate. Reduction was directly related to the amount of gastric juice, whereas it was poorly affected by varying the initial concentration of Cr(VI). At physiological temperature, the reaction was rather rapid, the top levels of reduction being attained after 10–20 min, followed by a plateau. Heating of gastric juice (5 min at 100°C) did not significantly affect its reducing ability (data not shown).

Fig. 1 provides examples of the circadian variations in gastric juice pH and in its Cr(VI)-reducing ability for 5 patients monitored for 24 consecutive hours. The reported pH values are those recorded in the intragastric environment at the moment of aspiration of the sample. The examples shown are representative of different circadian pH profiles, which are those typical for a healthy individual (No. 1), for duodenal ulcer patients, either untreated (No. 3) or receiving an antisecretory drug at dinner (No. 5) or at bedtime (No. 9), and for a colecystectomized patient (No. 15) suffering from dyspepsia and duodenal refluxes, with marked pH elevations after eating. The bulk of Cr(VI) reduction could be consistently detected after each meal. The maximum peaks, which were of a similar order of magnitude (40–60 μg/ml) for all monitored subjects, were generally attained 2–3 h after meal, when the buffering capacity of food was exhausted and pH was decreased to normal levels. No post-prandial peak could be detected only in the 3 patients receiving famotidine at dinner, which resulted in a rapid and persistent elevation of intragastric pH. At distance from meals, Cr(VI) reduction was less pronounced and independent of pH.

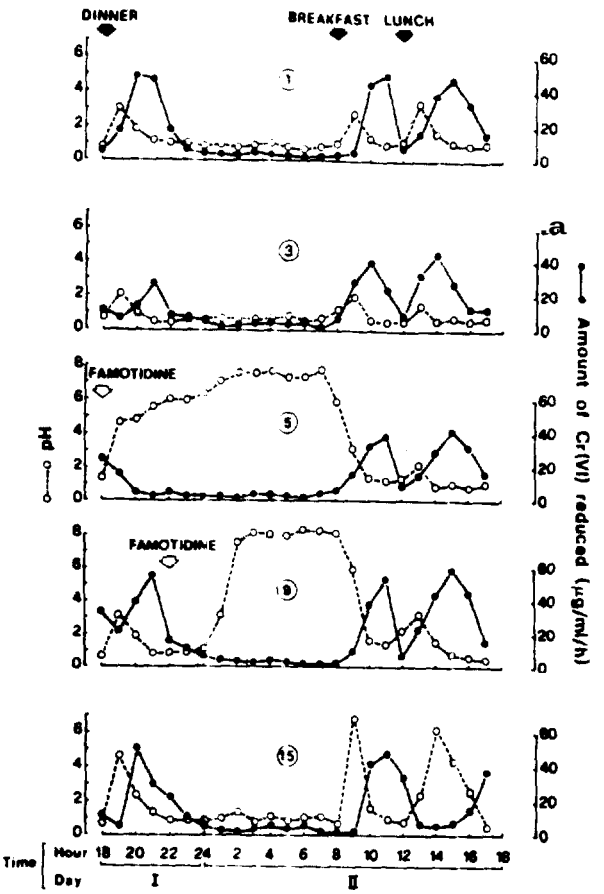

Fig. 1. Circadian monitoring of pH and Cr(VI) reduction by gastric juice from 5 patients (see the text for their identification).

The influence of pH was further investigated by artificially varying the reaction of gastric juice. Neutralization to pH 7.0 of 3 post-prandial samples, having an original pH ranging between 0.81 and 1.45, resulted in a statistically significant ($P < 0.001$) loss of their Cr(VI)-reducing ability (from a mean ± SD of 45.3 ± 4.0 to 9.7 ± 3.1 μg/ml). On the other hand, acidification to pH 1.0 of 3 samples having an original pH ranging between 5.1 and 7.2 only resulted in a slight and non-significant ($P > 0.05$) enhancement of their Cr(VI)-reducing ability (from 4.6 ± 1.9 to 6.3 ± 2.4 μg/ml).

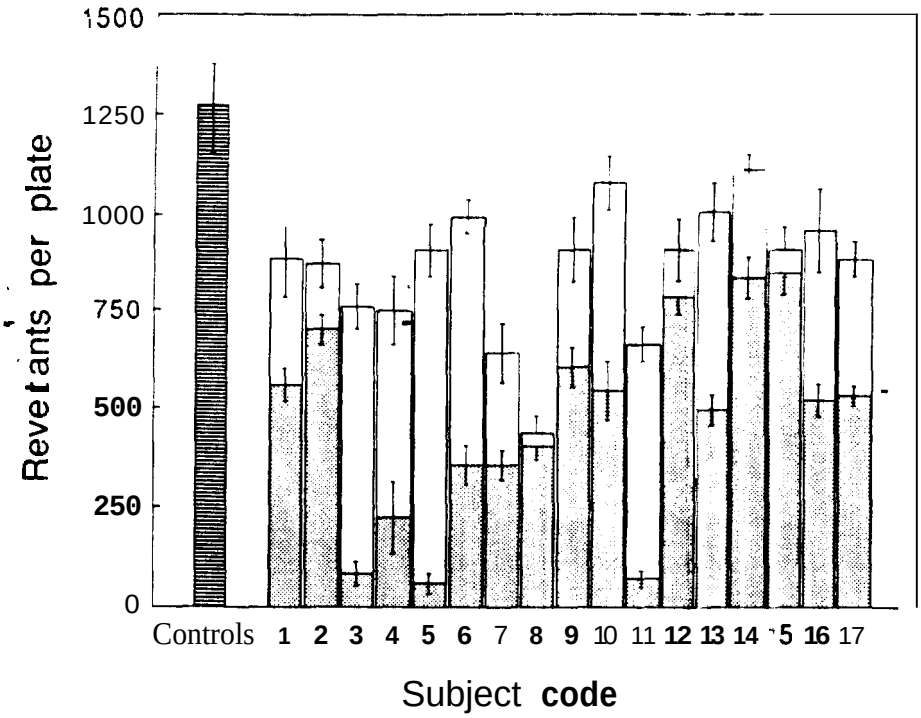

Fig. 2. Mutagenicity of sodium dichromate (28.6 μg, corresponding *to* 10 μg Cr(VI)/plate in strain TA102 of S. *typhimurium*, in the presence of 100 μl either of distilled water (controls) or of gastric juice samples collected from 17 subjects ai 7 a.m. (empty columns) or at 2 p.m. (dotted columns). The values represent the means ± SD of triplicate plates. Spontaneous revertants and revertants observed in the presence of each gastric juice sample were subtracted from those scored in chromium-containing plates.

Two gastric juice samples from each one of the 17 patients under scrutiny, i.e. under fasting conditions (collected at 7 a.m.) and 2 h after lunch (at 2 p.m.), were compared for their ability to decrease the mutagenicity of Cr(VI) in **S. *typhimurium*** (Fig. 2). It is noteworthy that, as shown in a separate study (De Flora et al., 1987), the same samples, without any addition of Cr(VI), yielded a weak increase in TA102 revertants only after lunch, which was taken into account in the analysis of the data. On the whole, the inhibitory effect of gastric juice at 2 p.m. (2.62 ± 1.36-fold decrease in revertants, as compared to Cr(VI) controls) was significantly greater ($P < 0.01$) than the one detected at 7 a.m. (1.46 ± 0.29-fold decrease). As shown by the magnitude of SD values, the samples of fasting individuals were rather homogeneous in activity, whereas a large variability was recorded among the post-prandial samples. The small size of patient groups did not allow us to evaluate the' influence of diagnosis and treatments on the decrease of Cr(VI) mutagenicity, which in any case was not a primary objective of the present study.

The effect of stimulation of gastric acid secretion with pen agastrin was evaluared in 3 patients. The decrease of revertants, as compared to Cr(VI) controls, was 1.12 ± 0.31 for the basal gastric juice samples, and 1.21 ± 0.42, 1.56 ± 0.23, 1.74 ± 0.12 and 2.08 ± 0.19 tor samples collected 15, 30, 45 and 60 min, respectively, after the secretagogue stimulation.

Discussion

The results herein reported confirm that human gastric juice is quits efficient in reducing Cr(VI) and in decreasing its mutagenicity. Although reduction of chromium was favoured by the acidic environment, as also demonstrated by artificially varying the pH of gastric juice samples, the reaction appeared to strictly depend on the presence of

thermostable reducing agents in gastric juice. In fact, excepting a temporary inhibition in patients receiving famotidine at dinner, peaks of activity were consistently detected during the 3–4-h period after each meal, when gastric secretion is markedly stimulated by the presence of food (Malagelada et al., 1976). Since organic matter can accelerate Cr(VI) reduction in acidified media (Stollenwerk and Grove, 1985), some contribution of food components cannot be ruled out. However, it is noteworthy that stimulation of secretion with pentagastrin resulted in a marked and time-related increase in Cr(VI) reduction by gastric juice also in fasting individuals.

On the other hand, Cr(VI) reduction was less pronounced in fasting individuals and during the night, irrespective of pH variations consequent to administration of antiulcer drugs. The inhibitory effect of the post-dinner peak just after treatment with famotidine is in agreement with a previous study (Petrilli and De Flora, 1982), reporting that the reducing capacity of gastric juice is significantly lower in fasting individuals receiving early in the morning, 2 h prior to collection of the sample, other H_2 blockers, i.e. cimetidine and ranitidine. Vitamin C, which in vitro efficiently reduces Cr(VI) (Petrilli and De Flora, 1978a), did not further stimulate Cr(VI) reduction by gastric juice when given just before lunch, either alone or in patients treated with the prostaglandin analog misoprostol. This probably depended on dilution of ascorbic acid in gastric juice and especially on its binding to food components.

The profiles of circadian Cr(VI) reduction were rather similar in the-different patients monitored, with levels of few μg per ml of gastric juice during the night and interdigestive periods, and peaks of some tens μg after each one of the 3 daily meals. It has been evaluated that in a fasting individual the daily gastric secretion accounts for 1.0–1.5 l (Kirsner, 1974), but in the 4-h period after a meal an average amount of approximately 800 ml is secreted (Malagelada et al., 1976). In addition to the local secretion, the intragastric volume also results from a dynamic balance between ingested water, swallowed saliva [which also has some Cr(VI)-reducing ability (Petrilli and De Flora, 1982)] and emptying into duodenum. Although only a crude estimate is possible, our findings suggest that the total amount of chromium reduced by human gastric juice is in the range of several tens mg per day.

Such a reducing capacity is expected to represent an important protective barrier against chromium introduced by the oral route or swallowed following reflux from the respiratory tract. In fact, the stable reduced form, i.e. Cr(III), is very poorly absorbed by the intestine (Donaldson and Barreras, 1966; Langård, 1982). Interestingly, after oral administration of radioactive chromate, the concentration of chromium in tissues and urines was found to be greater in fasted than in non-fasted laboratory animals (MacKenzie et al., 1959), which correlates with our finding of an enhanced efficiency of Cr(VI) reduction in the stomach after eating. The efficiency of Cr(VI) reduction in the stomach may also contribute to explain the very low toxicity of Cr(VI) by ingestion, the lethal dose of Cr(VI) compounds in humans being estimated in the range of 1.5–16 g per person (Langård, 1980), as well as the lack of oral carcinogenicity of this metal species. In fact, there is no positive report of carcinogenicity of chromium in rodents, when administered by the oral route (IARC, 1980). Also, the Cr(VI) standard for drinking water, which is 50 μg/l in most countries (e.g. EEC countries, U.S.A. and Japan), appears to be largely precautionary at the light of our quantitative data on the reducing capacity of human gastric juice.

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