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ORIGINAL ARTICLE

Prospective Study

Profiling cellular bioenergetics, glutathione levels, and caspase activities in stomach biopsies of patients with upper gastrointestinal symptoms

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Abstract

AIM: To measure biochemical parameters in stomach biopsies and test their suitability as diagnostic biomarkers for gastritis and precancerous lesions.

METHODS: Biopsies were obtained from the stomachs of two groups of patients (n = 40) undergoing fiber-optic endoscopy due to upper gastrointestinal symptoms. In the first group (n = 17), only the corpus region was examined. Biopsies were processed for microscopic examination and measurement of mitochondrial O_2 consumption (cellular respiration), cellular adenosine triphosphate (ATP), glutathione (GSH), and caspase activity. In the second group of patients (n = 23), both corpus and antral regions were studied. Some biopsies were processed for microscopic examination, while the others were used for measurements of cellular respiration and GSH level.

RESULTS: Microscopic examinations of gastric corpus biopsies from 17 patients revealed normal mucosae in 8 patients, superficial gastritis in 7 patients, and chronic atrophic gastritis in 1 patient. In patients with normal histology, the rate (mean ± SD) of cellular respiration was 0.17 ± 0.02 μmol/L O_2 min^{-1} mg^{-1}, ATP content was 487 ± 493 pmol/mg, and GSH was 469 ± 98 pmol/mg. Caspase activity was detected in 3 out of 8 specimens. The values of ATP and caspase activity were highly variable. The presence of superficial gastritis had insignificant effects on the measured biomarkers. In the patient with atrophic gastritis, cellular respiration was high and

ATP was relatively low, suggesting uncoupling oxidative phosphorylation. In the second cohort of patients, the examined biopsies showed either normal or superficial gastritis. The rate of cellular respiration (O_2. μmol/L min^{-1} mg^{-1}) was slightly higher in the corpus than the antrum (0.18 ± 0.05 *vs* 0.15 ± 0.04, P = 0.019). The value of GSH was about the same in both tissues (310 ± 135 *vs* 322 ± 155, P = 0.692).

CONCLUSION: The corpus mucosa was metabolically more active than the antrum tissue. The data in this study will help in understanding the pathophysiology of gastric mucosa.

Key words: Stomach; Gastritis; Mitochondria; Gastric mucosa; Cellular respiration

Core tip: Using small gastric mucosal biopsies obtained from patients with upper gastrointestinal symptoms, several cellular bioenergetic and dynamic parameters were measured and correlated with the histopathological features of the gastric mucosa.

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INTRODUCTION

The gastric mucosa of the normal human stomach includes numerous tubular epithelial glands. In the corpus region, each gland is lined by a heterogeneous population of cells secreting mucus, acid, pepsinogen, and various hormones and peptides[1]. In the antrum, the glands produce mainly mucus, hormones, and peptides. Analysis of gastric mucosal tissues from patients undergoing endoscopic examination (for recurrent upper gastrointestinal symptoms) and comparing them with gastric cancer tissues obtained from three different regions (safe margin, tumor edge, and tumor center) revealed that these tissues represent the multistep process of gastric carcinogenesis[2]. The sequential changes in the morphology of the gastric glands coincide with increased proliferating stem/progenitor cells during progression from normal to gastritis, into metaplasia, and finally into adenocarcinoma. Indeed when a stem cell-specific marker (Oct4) was used, the labeling pattern and the measurement of Oct4 protein content supported the central role of stem cells in driving precancerous and cancerous changes[3]. Since proliferation of gastric stem/progenitor cells and alteration of cellular dynamics is an important event in carcinogenesis, measurement of the cellular bioenergetics of gastric mucosal biopsies would be an emerging need.

Cellular bioenergetics reflects the biochemical processes involved in the energy metabolism (energy conversion or transformation). Cellular respiration implies the delivery of O_2 and metabolic fuels to the mitochondria, the oxidation of reduced metabolic fuels with passage of electrons to O_2, and the synthesis of adenosine triphosphate (ATP)[4]. Impaired bioenergetics therefore entails disturbances in these processes.

Cellular mitochondrial O_2 consumption is a highly sensitive biomarker for detecting tissue derangements[5]. Impairments in cellular membranes, mitochondria, or metabolic enzymes are expected to disrupt energy kinetics within the cell. Cells with intact bioenergetics are more capable of repairing damage. Furthermore, apoptosis with activation of caspases is more likely to result in cell death if associated with impaired cellular bioenergetics[6]. Therefore, energy metabolism has a significant impact on the fate of the cell. This notion stems from the dependency of human biological systems on aerobic metabolism. Cancer cells, on the other hand, may survive on anaerobic metabolism, a phenomenon commonly referred to as aerobic glycolysis or the Warburg effect[7].

Several human and animal studies have demonstrated that bioenergetics of the gastric epithelium are affected by various diseases (*e.g.*, ischemia) and toxins (*e.g.*, acetylsalicylic acid and non-steroidal anti-inflammatory drugs)[8-11]. Similarly, gastric tissue deficient in superoxide dismutase (a parietal cell enzyme that prevents the accumulation of superoxides) has mitochondrial dysfunction and perturbed energy metabolism, which manifests *via* reduced ATP and increased apoptosis[9].

Cellular bioenergetics has been used as a biomarker for metabolic diseases[12]. In the present study, cells and tissues obtained from patients were used to diagnose impaired cellular bioenergetics. The main aim of the present study was to show the feasibility of performing the same measurements [cellular respiration, ATP, glutathione (GSH), and caspase activity] on small gastric mucosal biopsies. The results here demonstrate the feasibility of measuring cellular mitochondrial O_2 consumption, ATP, GSH, and apoptosis in small mucosal biopsies from the stomach of patients.

MATERIALS AND METHODS

Materials

Pd(Ⅱ) complex of *meso*-tetra-(4-sulfonatophenyl)-tetrabenzoporphyrin (Pd phosphor) was purchased from Porphyrin Products (Logan, UT). Monobromobimane (mBBr, MW 271.111) was purchased from Molecular Probes (Eugene, Oregon). A lyophilized powder of caspase inhibitor I [*N*-benzyloxycarbonyl-Val-Ala-Asp (O-methyl)-fluoromethylketone; zVAD-fmk; MW 467.5; pan-caspase inhibitor] was purchased from Calbiochem (La Jolla, CA). Ac-DEVD-AMC (*N*-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin; MW 675.64; caspase-3

Table 1 Gastric corpus histology and measured biomarkers in the first cohort of patients (n = 17)

Patients	Age (yr)	Gender	Medications	Clinical findings	*H. pylori*	Histology	k_c	ATP	GSH	AMC
1	48	F	Mebeverine	Hysterectomy	-	Normal	0.18	1547 ± 5.6	396	0
2	38	F	PPI Thyroxin	Peptic ulcer Thyroid cancer	ND	Normal	0.19	13 ± 0.4	404	0
3	52	F	PPI NSAID Progesterone	IBS Thyroid neoplasm	+	Normal	0.13	267 ± 0.3	517	0
4	20	F	-	Hiatal hernia	-	Normal	0.18	492 ± 5.0	570	0
5	50	M	PPI, Losartan, Prednisolone	Acromegaly Hypertension	-	Normal	0.16	336 ± 3.8	360	0
6	34	M	Morbid obesity	Morbid obesity	+	Normal	0.16	495 ± 7.0	638	9
7	23	M	PPI Aspirin	Hypertension Dyslipidemia	-	Normal	0.18	14 ± 1.2	461	36
8	52	F	PPI Calcium, vitamin D Atorvastatin	-	-	Normal	0.19	731 ± 5.2	403	63
mean ± SD	40 ± 13						0.17 ± 0.02	487 ± 493	469 ± 98	
9	38	F	antacid	Mesenteric cyst	-	Superficial gastritis	0.15	1525 ± 8.7	476	95
10	22	M	PPI	-	+	Superficial gastritis	0.20	90 ± 4.1	347	14
11	46	F	PPI	-	-	Superficial gastritis	0.18	949 ± 1.3	373	14
12	28	F	PPI	IBS Hyperthyroidism Depression	+	Superficial gastritis	0.18	11 ± 0.2	830	13
13	65	M	PPI	-	-	Superficial gastritis	0.21	14 ± 0.9	496	0
14	62	F	PPI	-	+	Superficial gastritis	0.14	56 ± 2.0	366	0
15	55	F	PPI Tamoxifen	Breast cancer	-	Superficial gastritis	0.17	ND	ND	ND
mean ± SD	47 ± 18						0.18 ± 0.04	370 ± 563	481 ± 182	
16	72	F	PPI	Diabetes mellitus Hypertension Dyslipidemia Breast cancer	+	Atrophic gastritis	0.27	275 ± 4.2	606	2
17	36	M	-	Morbid obesity	+	Inadequate	0.14	37 ± 0.1	1138	105

kc, in μmol/L O_2 min^{-1} mg^{-1}; GSH in pmol/mg; ATP in pmol/mg; AMC: Peak area in arbitrary unit/mg × 10^3. ND: Not detectable; IBS: Irritable bower syndrome; NASID: Non-steroidal anti-inflammatory drugs; PPI: Proton pump inhibitors; *H. pylori*: *Helicobacter pylori*.

substrate) was purchased from Axxora LLC (San Diego, CA). Recombinant human active caspase-3 was purchased from BD Pharmingen™ (Becton Dickinson & Company, Franklin Lakes, NJ, United States). Glucose, 5,5'-dithio-bis(2-nitrobenzoic acid) [DTNB, MW 396.35, molar extinction coefficient at 412 nm 13.6 × 10^3], GSH (MW 307.43; pK_a 8.7), HPLC-grade methanol, dichloromethane, trifluoroacetic acid (TFA), methanesulfonic acid (MSA), and remaining reagents were purchased from Sigma-Aldrich (St. Louis, MO).

GSH was prepared in dH_2O and its concentration was measured by Ellman's reagent[9]. The GS-bimane derivative (GSH standard), sodium methane sulfonate (NaMS), mBBr, and DTNB solutions were prepared and stored as described[13-15]. DTNB working solution was 0.2 mmol/L DTNB in 100 mmol/L Tri-Cl (pH 8.0). GSH standard (2 μmol/L) was used to generate a calibration curve with each analytical run, which was linear from 10 to 200 picomoles ($R \geqslant 0.982$).

zVAD-fmk (2.14 mmol/L) and Ac-DEVD-AMC (7.4 mmol/L) were prepared in dimethyl sulfoxide and stored at -20 ℃. Pd phosphor (2.5 mg/mL = 2 mmol/L), sodium cyanide (CN, 1.0 mol/L), and glucose oxidase (10 mg/mL) were prepared in dH_2O and stored at -20 ℃.

Ethics

This work is compliant with the Declaration of Helsinki (2000) of the World Medical Association. The study was approved by the Institutional Review Board for the protection of human subjects, Al Ain Medical District Human Research Ethics Committee (Protocol No. 12/49 CRD 199). All patients provided informed written consent.

Gastric biopsies

The first cohort involved 17 patients who were admitted to the Endoscopy Unit of Tawam Hospital (Al Ain City, Abu Dhabi) for diagnostic fiber-optic endoscopy due to recurrent upper gastrointestinal symptoms (dyspepsia, abdominal pain, and heartburn) (Table 1). After collecting samples for standard patient care, five to eight additional mucosal biopsies were collected for the purpose of this

Table 2 Measured biomarkers in gastric corpus *vs* antrum in the second cohort of patients (n = 23)

Patients	Age (yr)	GI presentation	Gender	Gastric corpus		Gastric antrum	
				k_c	GSH	k_c	GSH
18	27	Liver lesion	F	0.11	357	0.12	378
19	71	Hypertension, diabetes, dyslipidemia, dyspepsia	M	0.13	627	0.16	379
20	22	Dyspepsia	M	0.13	366	0.25	331
21	18	Dyspepsia	F	0.14	319	0.19	260
22	30	Dyspepsia	M	0.16	733	0.21	853
23	34	Thyroidectomy, dyspepsia	F	0.14	449	0.18	408
24	24	Familial Mediterranean fever, dyspepsia	M	0.17	256	0.14	243
25	66	Diabetes, gastritis	M	0.15	243	0.12	317
26	69	Prostate cancer, aortic aneurysm, dyspepsia	M	0.22	269	0.13	470
27	22	Morbid obesity, dyspepsia	M	0.15	307	0.13	256
28	30	Dyspepsia	M	0.15	351	0.17	347
29	46	Obesity, dyspepsia	F	0.22	256	0.14	356
30	21	Dyspepsia	F	0.15	260	0.12	342
31	49	Hypertension, diabetes, ovarian, and cervical cancers, dyspepsia	F	0.18	202	0.10	104
32	44	Hypertension, diabetes, dyslipidemia, dyspepsia	F	0.18	180	0.17	166
33	34	Dyspepsia	F	0.20	218	0.11	256
34	18	Thalassemia major, s/p BMT, dyspepsia	F	0.15	219	0.12	193
35	81	GERD, esophagitis, hiatal hernia	M	0.19	279	0.11	219
36	62	Dyspepsia	F	0.28	258	0.19	554
37	40	Morbid obesity, dyslipidemia, chronic renal failure, dyspepsia	F	0.13	301	0.11	326
38	26	Data not available	F	0.30	305	0.18	177
39	39	Data not available	M	0.23	214	0.13	260
40	32	Data not available	F	0.21	166	0.12	211
mean ± SD	40.4 ± 19.8			0.18 ± 0.05	310 ± 135	0.15 ± 0.04	322 ± 155

kc, in μmol/L O_2 min^{-1} mg^{-1}; GSH: Glutathione, in pmol/mg; s/p. BMT: Status post bone marrow transplantation; GERD: Gastroesophageal reflux disease; GI: Gastrointestinal.

study. The samples (7.7-30 mg) varied in dimensions from 1 mm × 1 mm to 2 mm × 3 mm. Tissue samples were processed for histological examination and measurements of cellular respiration, caspase activity, ATP, and GSH. Values of the measured biomarkers were expressed per specimen as wet weight (in mg). For consistency, studied samples were obtained from the gastric corpus (body) midway along the greater curvature. In a separate cohort of 23 patients, samples were obtained from the corpus and the antrum; these additional samples were processed for histology, cellular respiration, and GSH only (due to limited sample availability) (Table 2).

For histological examination, tissue samples were processed as previously described[2]. *Helicobacter pylori* (*H. pylori*) infection was detected using Warthin-Starry stain[16] or urease-based test (campylobacter-like organism test, Ptonto DryTM, Medical Instruments Corporation, Brignais, France).

Within 20 min of sample collection, the specimens were transferred to 1.0 mL RPMI containing 0.5% fat-free bovine albumin and 3 μmol/L Pd phosphor and processed for O_2 measurements at 37 ℃ as previously described[17-19].

For measuring cellular ATP, a specimen from each patient was immediately homogenized in 0.5 mL of ice-cold 2% trichloroacetic acid for 2 min. The supernatants were collected by centrifugation (1000 *g* at 4 ℃ for 5 min) and stored at -20 ℃ until analysis as previously described[17-19].

For GSH labeling with mBBr, the reaction solution containing the gastric specimen (7.7-30 mg) was incubated at 25 ℃ for 15 min. The reaction was stopped with 100 μL of 70% perchloric acid and diluted with 400 μL of 10 mmol/L Tris-MSA. The tissue was vortexed, homogenized, and centrifuged. The supernatant was stored at -20 ℃ until HPLC analysis[13-15].

For measuring caspase activity, two specimens from each patient were used. They were immediately placed in 1.0 mL RPMI containing 37 μmol/L Ac-DEVD-AMC with and without 32 μmol/L zVAD-fmk as previously described[17-19].

HPLC

The reversed-phase HPLC system (Waters, Milford, MA, United States) was used. Ultrasphere IP column, 4.6 mm × 250 mm (Beckman, Fullerton, CA, United States) was operated at 25 ℃ at 1.0 mL/min. For GSH determination, solvent A was 0.1% (v/v) trifluoroacetic acid/water and solvent B was HPLC-grade methanol. The flow rate was 1.0 mL/min. The employed gradient was: 0 min, 10% B; 5 min 10% B; 13 min, 100% B; 15 min, 10% B; 20 min, re-inject. The excitation and emission wavelengths were 390 nm and 480 nm, respectively. The injection volume was 50 μL.

For AMC detection, the excitation wavelength was 380 nm and the emission wavelength 460 nm. Solvents A and B was HPLC-grade methanol: dH_2O 1:1 (isocratic). The run time was 15 min and the injection volume was 50 μL.

Statistical analysis

Data were analyzed using SPSS statistical package (version 19). The nonparametric Mann-Whitney test (2 independent variables) was used to compare samples.

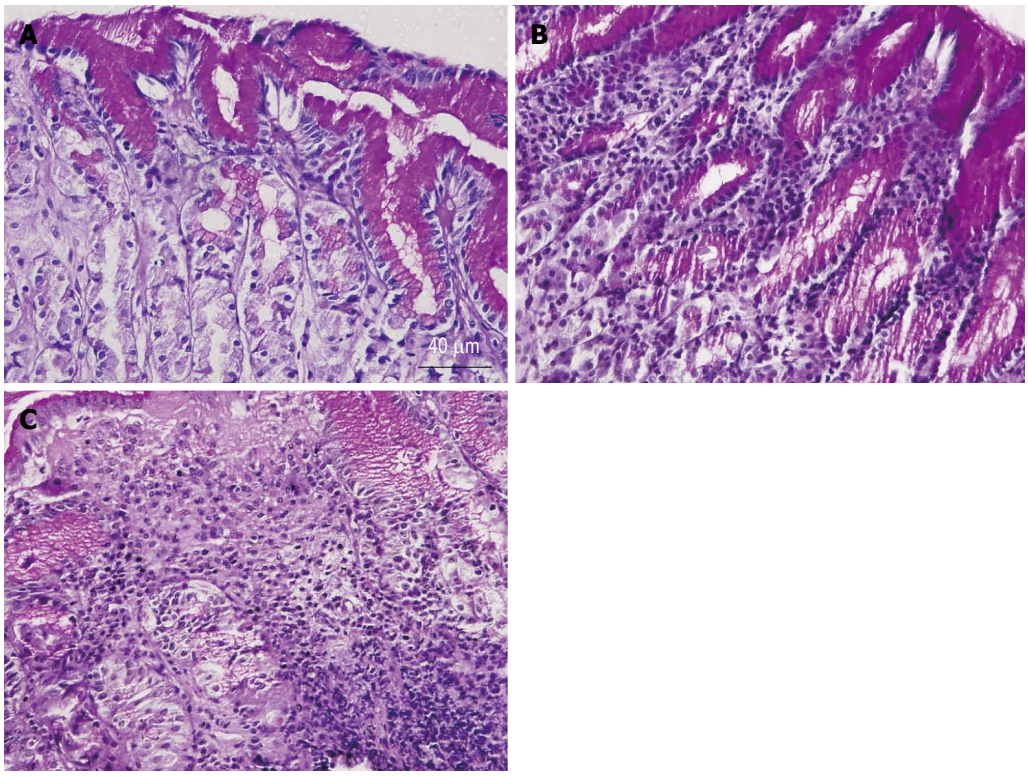

Figure 1 Representative micrographs of gastric corpus mucosal sections showing normal mucosa (A), superficial gastritis (B), and chronic atrophic gastritis (C). Note the mild infiltration of the gastric mucosa by lymphoid cells near the luminal surface in superficial gastritis (B) and the massive infiltration of the mucosa by lymphoid cells in atrophic gastritis (C).

RESULTS

Gastric corpus specimens were collected from the first 17 patients; their results are summarized in Table 1. The patients' age averaged 44 ± 16 years; 11 patients (65%) were females. All patients had recurrent upper gastrointestinal symptoms (dyspepsia, abdominal pain, and heartburn). Twelve (71%) patients were receiving proton pump inhibitors (PPI). Biopsies of 7 patients (41%) tested positive for *H. pylori* (Table 1). Microscopic examination of 5-micron-thick gastric mucosal sections revealed that 8 patients had normal gastric mucosa (Figure 1A). The biopsies of 7 patients had chronic superficial gastritis with infiltration of the luminal side of the mucosa with some inflammatory cells (Figure 1B). The gastric mucosa of only one patient (Patient 16) revealed evidence of chronic atrophic gastritis with massive infiltration with inflammatory cells (Figure 1C). The biopsy of one patient was inadequate for microscopic examination.

O_2 consumption by the stomach biopsy of Patient 16 (a patient with atrophic gastritis) is shown in Figure 2A. The rate of cellular respiration was the highest (0.27 μmol/L O_2 mg^{-1} min^{-1}), but cellular ATP was below the average (275 pmol/mg) (Table 1). This result suggested uncoupling oxidative phosphorylation (a state of high mitochondrial O_2 consumption with low cellular ATP) as a mechanism of the enhanced respiration. O_2 consumption was completely inhibited by cyanide, confirming that the oxidation occurred in the mitochondrial respiratory chain. The addition of glucose oxidase (which catalyzes the reaction of *D*-glucose + O_2 to *D*-glucono-δ-lactone + H_2O_2) depleted the remaining O_2 in the solution.

The rates of cellular mitochondrial O_2 consumption (k_c, μmol/L O_2 mg^{-1} min^{-1}) were 0.17 ± 0.02 for the 8 normal histology patients and 0.18 ± 0.03 for the 7 superficial gastritis patients (P = 0.867). The corresponding values for cellular ATP were 487 ± 493 and 370 ± 563, respectively (P = 0.573). The large variation in cellular ATP was likely due to sample processing. Nevertheless, the data show that superficial gastritis was not associated with bioenergetic changes in the gastric mucosa.

Representative GSH standard HPLC run and GSH standard curve are shown in Figure 2B; of note, GSH labeling with mBBr was blocked by N-ethylmaleimide (data not shown). Representative GSH run of acid-soluble supernatant of the stomach biopsy of Patient 10 (a patient with superficial gastritis) is shown in Figure 2C. Cellular GSH for the 8 patients with normal histology was 469 ± 98, and 481 ± 182 for the 7 patients with superficial gastritis (P = 0.662) (Table 1). Consistently, superficial gastritis was not associated with GSH changes in the gastric mucosa.

Ac-DEVD-AMC cleavage by the recombinant human active caspase-3 is shown in Figure 2D. The reaction, in 1.0 mL RPMI, contained 100 ng caspase-3 with and without 32 μmol/L zVAD-fmk (pan-caspase inhibitor). The mixtures were incubated at 37 ℃ for 10 min. Ac-DEVD-AMC (37 μmol/L) was then added and the incu-

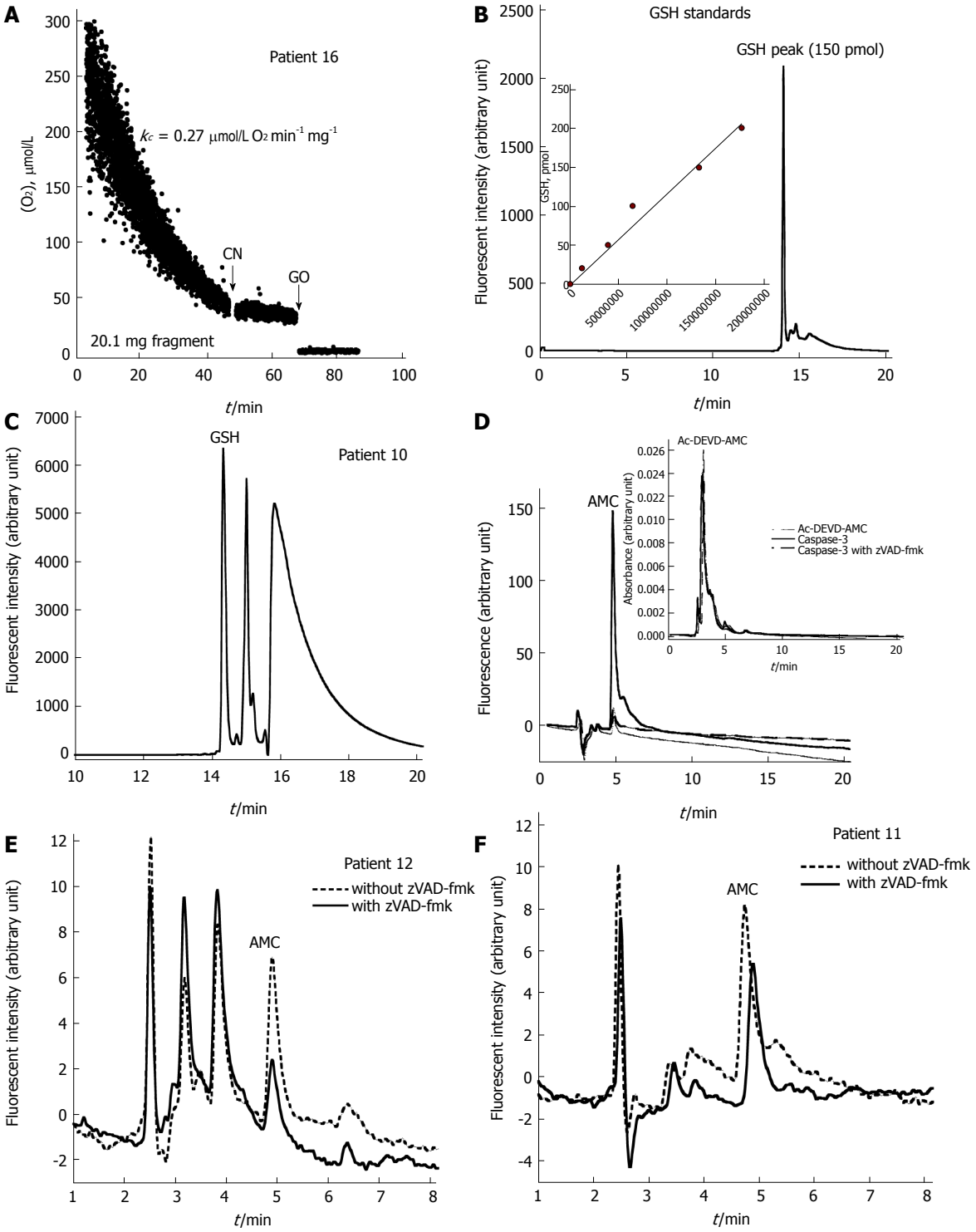

Figure 2 Representative measurements of gastric corpus cellular respiration, glutathione, and caspase activity. A: A run of cellular mitochondrial O_2 consumption by the gastric mucosa of Patient 16. The rate of respiration (k, μmo/L O_2 min^{-1}) was set as the negative of the slope of [O_2] *vs t*. The value of k_C (μmo/L O_2 min^{-1}) and the additions of 10 mmo/L cyanide (a specific inhibitor of cytochrome oxidase) and 50 μg/mL glucose oxidase (catalyzes the reaction of *D*-glucose + O_2 to *D*-glucono-δ-lactone + H_2O_2) are shown. O_2 consumption was inhibited by cyanide, confirming the oxidation occurred in the mitochondrial respiratory chain. O_2 was depleted by the addition of glucose oxidase, confirming the presence of dissolved O_2; B: A representative HPLC run of 150 pmol glutathione (GSH) standard (GSH retention time = 14.2 min); GSH standard curve is also shown [insert; GSH (pmol) = 0.00000117 x GSH peak area]; C: A representative HPLC run of cellular GSH in a stomach biopsy (23.9 mg mucosal fragment) from Patient 10. GSH peak area was 354508365 arbitrary units per 50 μL injection volume (reaction volume = 1.0 mL). Thus, cellular GSH content = 347 pmol mg^{-1} [(354508365 × 0.00000117 × 20)/23.9]; D: Representative HPLC runs for the Ac-DEVD-AMC cleavage reaction by human active caspase-3 with and without the pan-caspase inhibitor zVAD-fmk. The caspase-3 substrate Ac-DEVD-AMC was detected by absorbance at 380 nm with a retention time of about 3 min (insert). The product AMC was detected by fluorescence (380 nm excitation and 460 nm emission) with a retention time of about 4.8 min; E: Representative HPLC runs of caspase activities in the presence (solid line; 22.3 mg mucosal fragment) and absence (dashed line; 21.2 mg mucosal fragment) of the pan-caspase inhibitor zVAD-fmk for Patient 12. The AMC peak area without zVAD-fmk was 16015 arbitrary units/mg and with zVAD-fmk 2810 arbitrary units/mg. Intracellular caspase activity was set as AMC peak area without zVAD-fmk minus with zVAD-fmk, or 13205 (rounded down to 13 × 10^3) (Table 1); F: Representative HPLC runs of caspase activities with (solid line; 23.3 mg mucosal fragment) and without (dashed line; 19.1 mg mucosal fragment) zVAD-fmk for Patient 11. The AMC peak area without zVAD-fmk was 51207 arbitrary units/mg and with zVAD-fmk 37086 arbitrary unit mg^{-1}. Intracellular caspase activity, thus, was about 14 × 10^3 (Table 1).

bation continued at 37 ℃ for an additional 20 min. Ac DEVD-AMC was detected by absorbance at 380 nm with a retention time of approximately 3 min (Figure 2D). The product AMC was detected by fluorescence (380 nm excitation and 460 nm emission) with a retention time of about 5 min (Figure 2D). The cleavage reaction was inhibited by zVAD-fmk (Figure 2D). Caspase activity was set as the AMC peak area without zVAD-fmk minus the AMC peak area with zVAD-fmk. Representative HPLC runs of caspase-3 activity in the gastric corpus of Patients 12 and 11 are shown in Figure 2E and F, respectively. Caspase activity was detected in 3 of 8 (38%) patients with normal histology and 5 of 7 (71%) patients with abnormal histology (Table 1).

H. pylori had no significant effect on the rate of respiration, level of ATP, cellular GSH, or intracellular caspase activity ($P > 0.121$). Non-significant effects were also noted with respect to the use of PPI ($P > 0.104$).

The second cohort involved gastric corpus and antrum specimen collection from 23 additional patients. Due to limited sample availability, these biopsies were processed only for histology, cellular respiration, and GSH measurements (Table 2). Tissue samples for histology, however, were only available for 7 out of 23 (30%) patients; all had either normal or varying degrees of superficial (mild) gastritis. The patients' age averaged 40.4 ± 19.8 years; 14 patients (61%) were females. The rate of respiration (μmol/L O_2 mg^{-1} min^{-1}) was slightly higher in the corpus than the antrum (0.18 ± 0.05 *vs* 0.15 ± 0.04, $P = 0.019$). The value of GSH was about the same in both tissues (310 ± 135 *vs* 322 ± 155, $P = 0.692$).

DISCUSSION

Bioenergetic studies on the gastric epithelium are relatively limited, especially with respect to investigating human stomach diseases and the use of compound biomarkers[8,10,11,20-30]. The main purpose of this study was to examine the suitability of using biochemical parameters (cellular respiration, ATP, GSH, and caspase activity) as biomarkers for the gastric mucosa. The success of these measurements relies on the appropriate processing of the samples at the site of tissue collection. For O_2 measurements, the tissue should be immediately placed in ice-cold RPMI medium saturated with 95% O_2 and 5% CO_2. The sample should then be transferred to the laboratory on wet-ice and processed for the O_2 measurement within a few minutes of collection. For ATP, the tissue should be immediately quenched (at the procedure site) with acidic solution (freshly-made) to prevent ATP hydrolysis by cellular ATPases. For GSH, the tissue should be immediately immersed (at the procedure site) in thiol derivatization reaction that contains a large excess of mBBr (5 mmol/L). The GS-bimane derivatives are stable and can be stored until HPLC analysis. For caspase activity, the sample should be immediately placed in the Ac-DEVD-AMC cleavage reaction at the procedure site.

Having adhered to these experimental procedures, the values for the rate of cellular respiration (CV ≤ 17%) and GSH content (CV ≤ 48%) were reasonably consistent within the studied biopsies (Tables 1 and 2). These results were noted despite the wide-spectrum of clinical and histological variations among the patients and samples. Thus, cellular O_2 consumption and GSH are relatively preserved in the gastric mucosa. Cellular ATP (CV = 120%) and caspase activity (CV = 108%) were markedly varied however, likely due sample processing (Table 1).

We do identify that there are limitations to this study, as the sample size is relatively small and includes patients with minor gastric pathology. The clinical significance of these measurable biomarkers needs to be explored in future studies in patients with various pathologies, such as *H. pylori* infection, and the use of PPI.

Patient 3 had the lowest rate of respiration (0.13 μmol/L O_2 mg^{-1} min^{-1}); she had benign thyroid neoplasm and was taking multiple medications, including thyroxin, PPI, diclofenac, and medroxyprogesterone. Nevertheless, the cellular ATP, GSH, and caspase activity were not significantly different (Table 1).

Patient 16 had atrophic gastritis. Her rate of respiration was the highest (0.27 μmol/L O_2 mg^{-1} min^{-1}). She also had other complicated clinical problems (*e.g.*, diabetes mellitus, hypertension, dyslipidemia, and breast cancer) and was on PPI. While the rate of cellular respiration was the highest, the cellular ATP level was below average (275 pmol/mg) (Table 1), suggesting uncoupling oxidative phosphorylation.

Bioenergetics of the gastric epithelium was investigated in specimens collected from animal and human tissues[8,10,11,20]. In the bullfrog gastric mucosa, cellular mitochondrial O_2 consumption was increased and cellular ATP was decreased in the presence of acetylsalicylic acid[8]. Deficits in gastric cellular bioenergetics are also documented in shock and ischemia[10]. Non-steroidal anti-inflammatory drugs (NSAID) are shown to uncouple mitochondrial oxidative phosphorylation (lowering cellular ATP) in the gastric tissue[11]. Patient 6 was on aspirin and his cellular ATP was low (14 pmol/mg) (Table 1).

Activation of the mitochondrial apoptotic pathway is essential for *H. pylori*-induced apoptosis in gastric epithelial cells[21]. The *H. pylori* vacuolating cytotoxin A (vacA) causes direct mitochondrial disturbances and alterations in the bioenergetics of gastric epithelial cells[24]. Here, *H. pylori* had no noticeable effects on kc, ATP, GSH, or caspase activity. Nevertheless, the impact of *H. pylori* on the studied biomarkers requires a much larger sample size and appropriately selected control group.

Oxidative phosphorylation was measured in permeabilized corpus mucosal biopsies[22]. Cellular respiration was about 2-fold lower in patients with atrophic gastritis compared to non-atrophic gastritis. This effect was attributed to a deficiency of complex I of the respiratory chain[22]. Furthermore, limiting cellular bioenergetics was proposed to cause dysfunction of the zymogenic mucosal cells[23]. These studies demonstrate that stomach mucosal diseases can be associated with altered oxidative phosphorylation[23].

Activation of caspases permeabilizes (uncouples) the inner mitochondrial membrane, resulting in the collapse of the proton motive force, loss of electrochemical potential, and uncoupling of oxidative phosphorylation[25]. These processes lead to the rapid depletion of cellular nutrients, metabolic fuels, and ATP. The gastric mucosa is an intensely energy-consuming tissue. This demand is met by the mitochondria-rich acid producing parietal cells, which secrete the gastric acid and initiate the process of digestion. To prevent self-destruction, the columnar epithelium makes gastric mucosal barriers that resist the highly acidic and proteolytic gastric juice[26]. It is believed that mitochondrial dysfunctions impact gastric mucosal integrity, and thus measuring cellular mitochondrial O_2 consumption in gastric biopsies is justified.

Oxidative stress is induced in the stomach as a result of gastric insults, including chronic infections. GSH is a major detoxifying thiol which protects against oxidative stress. In indomethacin-treated rats, cellular GSH and mitochondrial enzymes are reduced. Esomeprazole, a proton pump inhibitor, was able to reserve GSH levels and mitochondrial enzyme activities[27]. Due to its γ-glutamyl transpeptidase, *H. pylori* can also reduce gastric epithelial GSH, exposing the bacterium, as well as the gastric epithelium, to oxidative stress[28].

ATP is produced in the mitochondria *via* oxidative phosphorylation by the proton-motive force that is used by ATP synthase to catalyze ADP phosphorylation[29]. The mitochondria are also the target of self-generated reactive oxygen species. Premalignant atrophic gastritis and gastric carcinoma are both associated with decreased respiratory capacity and mitochondrial complex I defici ency[22,30]. Therefore, investigating metabolic biomarkers in the gastric mucosa is much needed and future studies should determine whether they can be used to explore the mechanisms of diseases involving the gastric mucosa.

COMMENTS

Background

Since proliferation of gastric stem/progenitor cells and alteration of cellular dynamics are important events in carcinogenesis, the measurement of cellular bioenergetics of gastric mucosal biopsies would be an emerging need.

Research frontiers

Cellular bioenergetics has been used as a biomarker for some diseases. Whether it can be useful as a diagnostic tool for some gastric diseases is not known yet. In this study, the authors have demonstrated that various cellular bioenergetic and dynamic parameters could be measured and found useful for small gastric mucosal biopsies.

Innovations and breakthroughs

Recent reports have highlighted the importance of cellular dynamics and bioenergetics as diagnostic tools for some gastrointestinal and metabolic diseases. In this study, the authors report that cellular bioenergetics and other biochemical parameters could be useful tools for investigating stomach diseases.

Applications

By demonstrating the possible use of small mucosal biopsies for bioenergetic measurements, this study may represent a future strategy for the investigation and diagnosis of patients with upper gastrointestinal problems.

Terminology

Following microscopic examination, gastric mucosal biopsies were categorized as superficial (mild) or sever according to the Sydney classification criteria.

Peer review

This manuscript "Profiling cellular bioenergetics, glutathione levels, and caspase activities in stomach biopsies of patients with upper gastrointestinal symptoms" is very interesting study.

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