Human transforming growth factor \( \alpha \) (TGF-\( \alpha \)) is digested to a smaller (1–43), less biologically active, form in acidic gastric juice

T Marchbank, R Boulton, H Hansen, R J Playford

Background: Transforming growth factor \( \alpha \) (TGF-\( \alpha \)) is a 50 amino acid peptide with potent proliferative and cytoprotective activity present in gastric mucosa and juice.

Aims: To determine the forms and biological activity of natural and recombinant TGF-\( \alpha \) following incubation with acid pepsin.

Patients: Human gastric juice was obtained under basal conditions from patients taking acid suppressants and from volunteers undergoing intragastric neutralisation.

Methods: Samples were analysed using mass spectroscopy and/or high pressure liquid chromatography with radioimmunoassay. Biological activity was determined using thymidine incorporation into rat hepatocytes and an indomethacin/restraint induced gastric damage rat model.

Results: TGF-\( \alpha_{1–50} \) is cleaved to TGF-\( \alpha_{1–43} \) by acid pepsin and this is the predominant form in normal gastric juice. However, intragastric neutralisation or taking acid suppressants caused the predominant form to be TGF-\( \alpha_{1–43} \). TGF-\( \alpha_{1–43} \) had only half of the ability to maximally stimulate \([\text{H}]\text{thymidine incorporation} \) into primary rat hepatocytes (28 177 (1130) DPM/well for 2.16 nM TGF-\( \alpha_{1–50} \); v 63 184 (3536) DPM/well for TGF-\( \alpha_{1–43} \); p<0.001). A similar reduced potency was seen when used in an indomethacin induced rat gastric damage model (0.18 \( \mu \)mol/kg/h of TGF-\( \alpha_{1–43} \) reduced ulcer area by 19% whereas TGF-\( \alpha_{1–43} \) reduced area by 62%; p<0.001).

Conclusions: TGF-\( \alpha_{1–43} \) is cleaved to the TGF-\( \alpha_{1–43} \) form by acid pepsin, causing 2–5-fold loss of biological activity. Such changes may have relevance to the actions of acid suppressants and the importance of this peptide in both normal and abnormal growth.

Methods of analyses of samples to determine the forms of TGF-\( \alpha \)

Preparation of gastric juice samples prior to high pressure liquid chromatography (HPLC)

Samples of gastric juice which were to have HPLC performed were first concentrated using octadecylsilane (C18) cartridge chromatography, as described by Elson and colleagues.\(^{10}\) Eluates were then dried on a centrifugal evaporator (Savant; Farmingdale, New York, USA) and resuspended in 0.1% trifluoroacetic acid (TFA) prior to injection into the HPLC loop.

Reverse phase HPLC

Studies involving reverse phase HPLC used a Hewlett Packard 1100 (Stockport, UK) consisting of a quaternary pump delivery system, Rheodyne-7725 sample injector, and a 1 ml injection loop. The column used was an analytical “Jupiter” C5, 300 A, 3 \( \mu \)m (4.6x150 mm) column (Phenomenex UK Ltd). After application of the sample, the column was eluted isocratically (15% acetonitrile (AcN), 0.1% TFA) for 10 minutes before a gradient from 15–40% AcN, 0.1% TFA was run over 30 minutes. Samples were collected on a Fossy-Jr fraction collector and the system was controlled by a HP pentium PC with HP chemstation software. Samples were dried on a centrifugal evaporator and resuspended in water or Tris buffer prior to mass spectroscopy or radioimmunoassay, respectively.

MATERIAL AND METHODS

Materials

Recombinant and purified native human TGF-\( \alpha \) were obtained from Calbiochem (Nottingham, UK). Porcine pepsin was obtained from Sigma (P6887, Poole, UK) in the form of a lyophilised powder. All other chemicals were obtained from Sigma unless stated otherwise.
Radioimmunoassay (RIA) for TGF-α
TGF-α-like immunoreactivity was measured using a commercial RIA kit (BF-350; Biomedical Technologies Inc., Stoughton, Massachusetts, USA). Briefly, tubes were incubated for 24 hours at 4°C, and bound and free TGF-α were separated using 50 μl of donkey antiseep antibody and 50 μl of PEG followed by mixing and centrifugation at 2000 rpm for 15 minutes at 4°C. Typical results gave a maximal binding ratio (Tmax tubes) of 0.5 and a non-specific binding ratio (T, tubes) of 0.03. Sensitivity of the assay is 0.02 ng/tube. This radioimmunoassay does not cross react with epidermal growth factor (EGF).

Pilot study to determine the effect of acid pepsin digestion of TGF-α on immunoreactivity of TGF-α
Six aliquots of TGF-α were randomised to be incubated in either Tris buffer (pH 7) or 0.1 M HCl, each containing pepsin at 1 mg/ml, for one hour at 37°C. At the end of the incubation period all samples were neutralised using 0.1 M NaOH. Standard curves were then performed using all of these aliquots on a single day. Data were analysed by fitting three parameter logistic functions of loge concentration by least squares. These showed that the addition of acid pepsin. Fifty per cent of maximal binding occurred at a concentration of 5.2 ng/ml TGF-α in Tris/pepsin standards and 2.0 ng/ml TGF-α in standard which had been treated with acid pepsin. (significance of difference between 50% maximum binding values p<0.001). Concentrations of TGF-α in gastric juice samples were determined taking into account these small differences in immunoreactivity of the intact and digested forms.

Mass spectroscopy
Molecular weight was assessed by mass spectroscopy using the technique of matrix assisted laser desorption time of flight with a Finnigan LaserMAT mass spectrophotometer (San Jose, California, USA). Samples were mixed with 0.5 μl of alpha-cyano-4-hydroxycinnamic acid matrix (1% in 50% AcN, molecular weight 3496.9) and ubiquitin (molecular weight 8564.9). Our group have previously validated this technique for use in peptide sequence analyses.11

Methods of analyses of biological activity
In vitro assay
Background to method
Primary rat hepatocytes provide a robust reproducible method for determining the biological activity of EGF-like molecules. We have previously used this method to compare and contrast the relative bioactivity of different EGF receptor ligands.22 The methodology is therefore described only briefly below.

Isolation and culture of hepatocytes
Hepatocytes were isolated from male Wistar rats by in situ collagenase perfusion and cultured in Williams E medium using the method of Selden and Hodgson.23 Preliminary studies showed that the addition of Tris buffer alone, pepsin in Tris buffer, or acid and pepsin (which was subsequently neutralised) to the hepatocytes had no effect on their function, as determined by basal thymidine uptake, or their ability to respond to a standard dose of TGF-α added to the wells. Cell viability, determined by the ability to exclude 0.2% trypan blue, was greater than 80% in all experiments.

Thymidine incorporation
To assess the percentage of cells entering DNA synthesis, [3H]thymidine (2 μCi/well, 10 μl; Amersham International, Bucks, UK) was included in the cultures eight hours after the addition of test samples. The amount of [3H]thymidine incorporated was assessed biochemically, 18 hours after addition of thymidine.11 Cells were washed for 15 seconds with water using a Dynatech Multimash automatic cell harvester and solubilised by incubation at 37°C for one hour in 200 μl of 1 M KOH. Cell extract (50 μl) was counted in a β counter in 1 ml of scintillant (Optiphase Safe; LKB-Pharmacia, Bromma, Sweden).

Assay of biological activity of different forms of TGF-α in vivo
The ability of TGF-α to prevent gastric damage by indomethacin and restraint in rats was assessed using previously validated methods.14 Under light ether anaesthesia, rats (male Sprague Dawley, 225–250 g) had two subcutaneous cannulae inserted into the back of the neck and were then placed in Bullman restraint cages. Once the animals had recovered, a continuous subcutaneous infusion of saline or various doses and forms of TGF-α was started at 1 ml/h using a multi-syringe infusion pump (Harvard Apparatus, Massachusetts, USA). Thirty minutes later, 20 mg/kg of indomethacin were injected subcutaneously via the second cannulae. Animals were killed by stunning and cervical dislocation three hours later and their stomachs removed and inflated with 4 ml of 10% formalin. The next day the stomachs were opened and placed in fresh formalin prior to assessment. The stomachs were randomly coded and all analyses of gastric damage were assessed blind. Total ulcerated area (mm2/stomach) was assessed using a dissecting microscope (×10) with the aid of a square grid. The stomachs were then embedded in wax and the depth of damage assessed microscopically and given a microscopic ulcer score, as previously described.22 Using this system, each stomach was given a score of 0 to 4, with 0=no damage, 1=one small erosion (less than 0.5 mm), 2=two small or one large erosion (greater than 0.5 mm), 3=two or more large erosions, and 4=any area of ulceration extending to the muscularis mucosa.

Ethics approval
Local ethics approval was obtained for studies involving human volunteers and subjects gave informed consent.

Study protocols
Study 1: stability of recombinant TGF-α in acid pepsin in vitro
Aliquots of 10 μg of recombinant TGF-α were incubated in various solutions for one hour at 37°C. These were: (a) isotonic saline; (b) 0.1 M HCl; (c) Tris buffer (pH 7.4) containing 1 mg/ml of pepsin; and (d) 0.1 M HCl containing 1 mg/ml of pepsin. All assay conditions were performed in quadruplicate. One of the samples from each condition was assessed using mass spectroscopy without further separation. The other three samples underwent HPLC. Following HPLC separation, each fraction was split into two. One half of the fraction was analysed by RIA and the other by mass spectroscopy. In addition, to ensure the biological relevance of our studies, 5 μg aliquots of purified human TGF-α were incubated for one hour in saline or HCl plus pepsin and analysed in an identical fashion. To determine how fast the digestion of TGF-α occurred in acid pepsin, 5 μg of recombinant TGF-α were incubated in 1 ml of acid pepsin at 37°C for 10 minutes; this was then immediately neutralised to pH 7 using NaOH and analysed as above.

Study 2: form of TGF-α present in normal human acidic and neutral gastric juice
Baseline gastric juice samples were obtained by aspiration from patients who had a nasogastric tube inserted for clinical reasons. Samples from six patients taking proton pump inhibitors (PPIs) for clinical reasons were compared with samples obtained from six control patients. pH was determined for all samples to ensure control samples had a pH of 3 or less and PPI samples had a pH of more than 4. Samples were
screened for the presence of trypsin (using a standard spectrophotometric method employing Na-benzoyl-\textasciitilde[1]-L-arginine-p-nitroanilide) and bilirubin to ensure there was no contamination by pancreatic proteases. Samples that contained no detectable trypsin or bilirubin were frozen and stored at \(-20^\circ\text{C}\) prior to HPLC and RIA.

Study 3: changes in the form of intragastric TGF\(\alpha\) in response to intragastric neutralisation

Seven subjects took part in this study (six males, one female). After an overnight fast, a double lumen nasogastric tube was placed for continuous aspiration from the gastric antrum with infusion 20 cm proximally. For six subjects, the study comprised a 40 minute perfusion period with normal saline followed by 40 minutes of perfusion with 0.17 M sodium bicarbonate. The seventh subject had an 80 minute perfusion period with saline alone. Perfusion was at 5 ml/min and all perfusates contained 5 g/l of polyethylene glycol 4000 (subsequently assayed using a standard turbidimetric method) to allow for correction for duodenal losses. Aspirates were collected continuously on ice into a beaker containing a pH electrode to allow the juice to be continuously titrated to pH 7 using NaOH. Samples were mixed thoroughly and stored in 10 minute batches. At the midpoint of each collection period, a 1 ml sample was collected directly from the aspiration tubing to allow analysis of pH and pepsin activity. Neutralised samples which contained no detectable trypsin or bilirubin were frozen and stored at \(-20^\circ\text{C}\) prior to HPLC and RIA.

Study 4: biological effects of changes in the form of TGF\(\alpha\)

**Preparation of TGF\(\alpha\)**

To determine the effect of acid pepsin digestion on the biological activity of TGF\(\alpha\), four aliquots of 100 \(\mu\)g of previously pooled recombinant TGF\(\alpha\) were randomly allocated to be incubated for one hour at 37°C in either 1 ml of 0.1 M HCl containing 1 mg pepsin (that would be expected to digest TGF\(\alpha\)) or 1 ml of Tris buffer pH 7.0 containing 1 mg pepsin (that would not be expected to digest TGF\(\alpha\)). At the end of the incubation period, the stock solutions were neutralised to pH 7. Samples were separated by HPLC as previously described and fractions containing TGF\(\alpha_{1-43}\) and TGF\(\alpha_{1-50}\) identified by mass spectroscopy. Protein concentrations of the fractions were determined using a Biorad protein assay and samples were then diluted in Tris buffer containing 1 mg/ml bovine serum albumin to minimise adhesion to the container during storage. These solutions were then used to perform studies examining changes in bioactivity.

**In vitro assay**

Various concentrations (0–9 nM) of either intact TGF\(\alpha\) (Tris/pepsin treated) or the acid pepsin treated TGF\(\alpha\) were added to the hepatocytes and thymidine incorporation determined 26 hours later. For both intact and acid pepsin treated TGF\(\alpha\), each dose of TGF\(\alpha\) was measured in quadruplicate in four separate wells.

**In vivo gastric damaging study**

Rats were randomised to receive saline (containing bovine serum albumin 0.2 mg/ml), “intact” TGF\(\alpha\) (Tris-pepsin treated TGF\(\alpha\)) at either 0.18 or 0.90 \(\mu\)mol/kg/h, or acid pepsin treated TGF\(\alpha\) at either 0.18 or 0.90 \(\mu\)mol/kg/h.

**Statistics**

Data from the hepatocyte assay were analysed using the Prism 2.0 computer package. Analysis of variance followed by \(t\) testing was carried out: \(p<0.05\) was taken as significant.

The gastric damaging model was analysed using analysis of variance followed by \(t\) testing based on the mean square error and degrees of freedom obtained from the analysis of variance, as appropriate: \(p<0.05\) was taken as significant.

**RESULTS**

**Study 1: stability of recombinant TGF\(\alpha\) in acid pepsin in vitro**

Intact recombinant TGF\(\alpha\), purified TGF\(\alpha\), TGF\(\alpha\) which had been incubated in HCl without pepsin, and Tris pepsin treated TGF\(\alpha\) gave a single peak on mass spectroscopy corresponding to intact TGF\(\alpha_{1-43}\). Measured mass was always in the range 5546–5554 (expected molecular weight of TGF\(\alpha\) 5546). In samples that were further analysed by performing HPLC followed by RIA, a single peak of immunoreactivity was found in fraction 14 (fig 1A). Mass spectroscopy of aliquots of this fraction also gave a mass corresponding to TGF\(\alpha_{1-50}\).
TGF-α which had been incubated for one hour in acid pepsin analysed by mass spectroscopy without HPLC separation gave one peak corresponding to TGF-α<sub>1–43</sub> (measured molecular weight 4796, theoretical molecular weight 4796). HPLC separation followed by RIA showed one peak of immunoreactivity in fractions 11 and 12 (fig 1C). Mass spectroscopy of aliquots of these fractions also gave a mass corresponding to TGF-α<sub>1–43</sub>. Following 10 minutes of incubation of TGF-α with acid pepsin, approximately 40% of TGF-α remained intact (fig 1B) with 60% of TGF-α being in the TGF-α<sub>1–43</sub> form. TGF-α incubated in acid alone resulted in a peak in fraction 14 indicating intact TGF-α<sub>1–43</sub> (as in fig 1A). Studies using native purified TGF-α gave similar results (data not shown).

### Study 2: form of TGF-α present in normal acidic and neutral gastric juice

Gastric juice samples from control patients (pH <3) gave one peak corresponding to TGF-α<sub>1–43</sub>, which eluted in fractions 11 and 12, when analysed by HPLC and RIA. Gastric juice samples collected from two patients taking PPIs (pH >4) resulted in one peak in fractions 11 and 12, corresponding to TGF-α<sub>1–43</sub>. However, gastric juice samples collected from four patients taking PPIs (pH >4) resulted in a split peak eluting in fractions 12 and 14, corresponding to TGF-α<sub>1–43</sub> and TGF-α<sub>1–50</sub>, respectively, with approximately 40% of TGF-α eluting in the intact form. Total gastric juice TGF-α concentrations from the various subgroups were similar, giving a pooled value of 186 (41–839) ng/l (median (interquartile range)).

### Study 3: changes in the forms of intragastric TGF-α in response to intragastric neutralisation

Subjects receiving saline infusion all had a gastric pH of between 1.8 and 3.0. Prior to and during saline infusion, gastric samples showed that virtually all of the TGF-α eluted from the HPLC column in the position of TGF-α<sub>1–43</sub> (fig 2A).

During bicarbonate perfusion, the pH of the samples were all in the range 6.8–7.2 and the peak of TGF-α-LI corresponded to TGF-α<sub>1–43</sub> with a slight shoulder in the position of TGF-α<sub>1–50</sub> (fig 2B). The subject who had saline perfusion throughout showed no change in the form of TGF-α present during the final period (TGF-α<sub>1–43</sub>).

### Study 4: biological activity assays

[<sup>3H</sup>]Thymidine uptake into primary rat hepatocytes

Both forms of TGF-α stimulated thymidine uptake in hepatocytes in a dose dependent manner (fig 3). However, maximal uptake was seen with TGF-α<sub>1–50</sub> when compared to TGF-α<sub>1–43</sub> (p<0.01 for all doses greater than 0.54 nM).

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**Figure 2**  Influence of gastric pH on the forms of transforming growth factor α (TGF-α) present in gastric juice. TGF-α present in resting normal gastric juice (pH <3) or collected during a saline wash eluted from high pressure liquid chromatography as a single peak in fractions 11 and 12, corresponding to TGF-α<sub>1–43</sub> (A). Changing the intragastric infusion to sodium bicarbonate caused the pH to rise to approximately 7 and the predominant form of TGF-α to be the 1–50 form (fraction 14) (B).

**Figure 3**  Effect of acid pepsin pretreatment on the biological activity of transforming growth factor α (TGF-α) using hepatocytes as a bioassay. Recombinant TGF-α<sub>1–50</sub> was pretreated by incubation in either pepsin in neutral Tris buffer (preserving the 1–50 form) or acid pepsin (causing digestion to the 1–43 form), followed by neutralisation. The biological activity of the samples were then compared by determining their ability to stimulate [<sup>3H</sup>]thymidine incorporation into primary rat hepatocytes. Each concentration was tested in quadruplicate. Acid pepsin treated TGF-α (TGF-α<sub>1–50</sub>) only resulted in 50% of the maximal stimulation of intact TGF-α (TGF-α<sub>1–43</sub>). For all doses studied, biological activity was greater for the TGF-α<sub>1–50</sub> form versus the same dose of TGF-α<sub>1–43</sub> (p<0.01 for all doses greater than 0.54 nM).

**Figure 4**  Effect of acid pepsin pretreatment on the biological activity of transforming growth factor α (TGF-α) using attenuation of gastric damage as bioassay. Samples were treated as described in fig 3. Intact TGF-α<sub>1–50</sub> or acid pepsin treated TGF-α<sub>1–43</sub> was infused in various doses into animals that were restrained and given indomethacin [20 mg/kg]. The mean surface area of damage (SEM (mm<sup>2</sup>/stomach) is shown for each dose. Animals receiving intact TGF-α<sub>1–50</sub> had less gastric damage than those receiving acid pepsin treated TGF-α<sub>1–43</sub> (p<0.001); n=6–8 per group.
stimulation by TGF-α\(\alpha\)\textsubscript{29} was approximately double that caused by TGF-α\(\alpha\)\textsubscript{26–29} (76447 (5654) v 35375 (2710) DPM/well for 9 nM). For all doses studied, biological activity was greater for the TGF-α\(\alpha\)\textsubscript{26–29} form compared with the same dose of TGF-α\(\alpha\)\textsubscript{1–43} (p<0.01 for all doses greater than 0.54 nM).

**Gastric damaging model**

Both forms of TGF-α decreased the amount of gastric damage in a dose dependent manner compared with the control (saline) group (fig 4). Analysis of variance showed a significant effect of both the dose (\(F_{1,29}=13.238, p=0.0000\)) and form (\(F_{1,29}=20.597, p=0.000\)) of TGF-α. For both doses tested, TGF-α\(\alpha\)\textsubscript{1–43} was significantly more potent than the TGF-α\(\alpha\)\textsubscript{26–29} form in its ability to reduce injury (p<0.01, see fig 4). Assessment using the microscopical damage score gave similar results (data not shown). An absolute quantitative comparison of the relative potencies of the two forms of TGF-α is not possible in this study. However, the area of damage seen in animals receiving 0.9 \(\mu\)mol/kg/h of the TGF-α\(\alpha\)\textsubscript{26–29} was slightly higher than that seen in animals receiving 0.18 \(\mu\)mol/kg/h of TGF-α\(\alpha\)\textsubscript{1–43}, suggesting that, in this particular model, the intact form is about 3–5 times as potent as TGF-α\(\alpha\)\textsubscript{1–43}.

**DISCUSSION**

We have shown that TGF-α is susceptible to cleavage by acid peptin in vivo and vitro. Under normal basal acidic conditions, the predominant form in gastric juice is TGF-α\(\alpha\)\textsubscript{1–43} but following intragastric neutralisation or in most patients taking clinically relevant doses of PPIs, the predominant form in gastric juice becomes the full length (TGF-α\(\alpha\)\textsubscript{1–50}) form. We have also shown that the acid peptin digested TGF-α\(\alpha\)\textsubscript{1–43} has about half to one fifth of the biological activity of the intact TGF-α\(\alpha\)\textsubscript{1–50} molecule.

Mass spectroscopy provides a rapid sensitive method of determining the molecular weight of test peptides and is capable of demonstrating minor “clipping” of peptides which are not seen using simple size exclusion analyses. Mass spectroscopy is however not quantitative and is less useful in analysing peptides in complex biological solutions which may contain multiple peptides of similar molecular weight. We therefore used it in conjunction with HPLC and RIA to determine qualitative and quantitative changes in the forms of TGF-α.

For the in vitro studies, a variety of cells of gastrointestinal origin were available. Carcinoma cell lines of colon (for example, HT29) or gastric (for example, AGS) origin have previously been used by us and other groups to assess proliferative and antiproliferative activity of various peptides. These cells have the advantage of being derived from the human gastrointestinal epithelium and being relatively easy to culture. However, they also have the disadvantages of being obtained from carcinoma cell lines and, of particular relevance for the present study, of having a relatively flat dose-response curve. We therefore decided to use the rat hepatocyte system as it provides a robust reproducible model for examining growth factor activity and we have previous experience of its reliability in assessing the biological activity of other EGF receptor ligands.11 It provides a highly reproducible dose-response curve and is of particular value for examining growth factor activity in intestinal juice as hepatocytes are not adversely affected by normal small intestinal luminal contents.15 It cannot be used however to assess changes in cell number as under the conditions used, hepatocytes complete cell DNA synthesis but cell division occurs infrequently and no net increase in cell number is seen.16

There are many well validated acute models of gastric injury. We chose the indomethacin/restraint model as our in vivo assay system because peptic ulceration due to non-steroidal anti-inflammatory drugs is a major source of morbidity and mortality in humans2 and we have previously used it to examine the influence of peptic digestion on the EGF molecule.17

TGF-α is produced in the mucosa throughout the gastrointestinal tract and is synthesised as a 160 amino acid precursor molecule that spans the cell membrane. Subsequent exposure of the external domain to specific proteases releases the soluble mature 50 amino acid form18 which has a molecular weight of 5546 and includes three Cys-Cys double bonds. TGF-α shares 33% sequence homology with EGF and has nearly identical biological activity, acting through the same EGF receptor (EGF-R), also known as the erbB-1 receptor. There is continuing controversy as to whether erbB-1 receptors are present on the apical (luminal) surface in addition to the basolateral membrane of the normal bowel.20,21 However, there is general agreement that luminal administration of erbB-1 ligands can stimulate growth and repair, acting via the luminal surface, when administered to the damaged bowel of both rats22 and humans.23

Our studies have shown that, under acidic conditions, peptin cleaved the “Cys-Glu bond of the TGF-α molecule. Several isoforms of peptin exist which differ slightly in their preferred substrate specificity and pH profile.24 However, we have previously shown that the vast majority of proteolytic activity is dependant on an acidic pH with proteolytic activity markedly diminishing when pH rises to 4 or above.25 Inactivation of peptin therefore almost certainly explains the finding of the change in predominant form to TGF-α\(\alpha\)\textsubscript{1–50} which was seen in patients taking PPIs or during intragastric neutralisation. Our in vitro study on the stability of native TGF-α in acid peptin gave similar results to those found using recombinant TGF-α. However, caution should always be shown before assuming recombinant peptides behave in an identical fashion to their native forms.

The concentration of TGF-α in gastric juice found in our studies (about 200 ng/l) is similar to that reported by others,26 being about one third of that of EGF (500 ng/l). The function of TGF-α in gastric juice is unclear although several lines of evidence suggest pathophysiological relevance: TGF-α is a potent stimulant of proliferation of several gastrointestinal cell lines in vitro,27 protects against injury and/or stimulates repair in several different animal models of gastrointestinal injury (for example, see Konturek and colleagues28 and Romano and colleagues29), and physiological concentrations of gastric juice TGF-α are probably sufficient to stimulate proliferation of human gastric explants.30 In addition, pathophysiological relevance for this concentration of TGF-α in gastric juice is supported by the finding that slaldenectomy of rats reduces the amount of EGF present in gastric juice by about 60% (approximately 300 ng/l), which is similar to the receptor ligand contribution from TGF-α (200 ng/l), causing an increased susceptibility to noxious agents with increased ulceration and delayed healing.27 Taken together, these results suggest an important role for gastric juice TGF-α, working in combination with EGF, in maintaining epithelial integrity. It is also important to note that virtually all previous studies measuring TGF-α in gastric juice have used immunoassays. Our finding that the immunoreactivity of the digested form is reduced is therefore important as it may lead to underestimation of gastric levels of TGF-α under acidic conditions unless appropriate corrections are made.

Indomethacin causes damage to the gastrointestinal tract by several mechanisms, including reduction of mucosal prostaglandin levels, reduction of mucosal blood flow, stimulating neutrophil activation, and possibly also stimulating apoptosis.31 It is likely that many of these mechanisms will be influenced by the presence of the TGF-α. Both forms of TGF-α significantly reduced indomethacin induced gastric damage when administered at both 0.18 and 0.9 \(\mu\)mol/kg/h. The lower dose of TGF-α probably did not affect gastric acid secretion12 but the higher dose may have done so because 1.8
μmol/kg/h of TGF-α,14 administered intravenously, decreases gastric acid secretion by 72%.15 This may be relevant to the mechanism by which TGF-α decreases gastric damage in our rat model as we have previously shown the damage to be acid dependent.16 Cleavage of the terminal seven amino acids of TGF-α caused a 3–5 fold reduction in its ability to reduce injury in this model, which was similar to the reduction in activity found using the in vitro assay. The results of the in vivo study are likely to be due to reduced interaction of circulating TGF-α,17 with its receptors on gut cells. Alteration in the circulating half-life of the truncated form remains a possibility but is much less likely in view of the comparable data from in vitro studies.

Tam and colleagues18 examined which areas of the TGF-α molecule played a key role in its interaction with the erbB-1 receptor. They found that the seven C terminal amino acids have a major influence on its ability to bind and stimulate mitogenesis of rat kidney fibroblasts, with TGF-α1–43, having only 1/1000 of the activity of TGF-α1–50. This is a much lower level of activity than that found in our studies although Tam et al did not perform any in vivo studies. Importantly, in their studies, TGF-α1–43, was produced using solid phase peptide synthesis, rather than recombinant technology, and the authors specifically mention the difficulties of obtaining correct refolding of TGF-α1–43, using this method. The different cell lines used (rat kidney fibroblasts vs primary rat hepatocytes) may also be relevant.

Recent studies examining the effect of knockout of EGF-R ligands suggest that EGF-R ligands function in a synergistic fashion with deletion of an individual member having relatively little effect on phenotype19 20 whereas multiple knockouts or deletion of the EGF-R itself resulting in profound effects on the gut.21 22 The current series of studies suggest that changing the size of the TGF-α molecule from the truncated form to TGF-α1–50, as found in most patients taking PPIs, is biologically equivalent to a 2–3 fold increase in TGF-α concentration. We have previously shown a similar reduction in gastric EGF bioactivity in response to acid and pepsin.23 The current findings therefore support the idea that luminal pH is likely to have a major influence on multiple luminal growth factors within the gastric juice. This may well have relevance to pro-healing activity (as seen with acid suppressants) and also conditions associated with abnormal growth, such as patients with pernicious anaemia or severe atrophic gastritis (who fail to make gastric acid) and are known to have an increased risk of development of gastric carcinoma.24 Further studies appear warranted.

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Bleeding peptic ulcer

We read with interest the paper on prediction of therapeutic failure after adrenaline injection plus heater probe treatment in patients with bleeding peptic ulcer by Wong and colleagues (Gut 2002;50: 322–5). Even though the authors qualified their generalisation, the statement that “elderly patients often succumb to their concomitant illnesses rather than the bleeding itself” needs to be challenged as being unnecessarily defeatist, given the fact that timeliness of surgical intervention and, as shown below, postoperative management at the intensive care level, may be more crucial to survival than comorbidity as such.

Case report

A 70-year-old woman with congestive cardiac failure (including radiographically validated left ventricular failure) and chronic obstructive airways disease experienced an episode of haematemesis and melena with an associated blood pressure of 78/48 mm Hg on the 1 March 2002, which was the eighth day of her hospital stay. Endoscopy revealed a large actively bleeding duodenal ulcer, which was managed with endoscopic haemostasis, rapidly followed by definitive laparotomy and under running of the bleeding vessel. She was then transferred to a neighbouring hospital for postoperative intensive care management, and this included a 24-hour period of artificial ventilation. On the 11th postoperative day, having been repatriated to our hospital, she was clinically much improved even though her arterial blood tensions while breathing room air were as follows: partial oxygen tension (PaO₂) 4.9 kPa (normal range 10–14), partial carbon dioxide tension (pCO₂) 6.9 kPa (normal range 4.5–6.1), and oxygen saturation 70%, with concurrent transcutable oxygen saturation 72% (normal range 95–98%). Her clinical status continued to improve on diuretics, angiotensin converting enzyme inhibitors, bronchodilators, and supplemental oxygen. On her 29th postoperative day, lung function tests revealed a one second forced expiratory volume (FEV₁) of 0.86 litres (40% predicted), forced vital capacity (FVC) of 1.59 litres (61% predicted), and an FEV₁/FVC ratio of 54% (typically less than 70% in airflow obstruction). She could now perform a modified version of the “shuttle” walk for a distance of 30 m briskly, without stopping for breath, and also without supplemental oxygen. Repeat arterial blood gas tensions on 30 March 2002 showed PaO₂ 7.7 kPa and PaCO₂ 5.8 kPa while breathing room air.

Comment

On the basis of age, comorbidity, shock at presentation, and endoscopic stigmata of recent haemorrhage, this patient had a high risk of death with or without surgical intervention. Only timely intervention and impeccable postoperative care could tip the scales in her favour, hence the successful outcome documented here.

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Department of Adult Medicine, Tameside General Hospital, Fountain Street, Ashton under Lyne OL6 9RW, UK

References


CORRECTIONS

The authors of Marchbank et al (Gut 2002; 51: 787–92) in the December issue of the journal, have noted a typographical error in their paper. In the results section, it should state that the pH of the two subjects who were taking proton pump inhibitors who did not show a change to the larger form of TGFα had a pH of less than, and not greater than 4 as published. The authors apologise for the error.

The following errors occurred in the paper “Prospective study of liver dysfunction in pregnancy in Southwest Wales” in the December issue by Ch’ng et al (Gut 2002; 51: 876–80) as final author corrections were not included. The published version does not clearly distinguish those patients in whom pre-eclampsia was the sole identifiable cause of liver dysfunction from those in whom pre-eclampsia co-existed with another cause such as HELLP syndrome, obstetric cholestasis, or sepsis. The corrections are to the abstract, tables 1, 2, and 3, and part of the text of the results section on page 878 under the sub-heading “Diagnoses accounting for abnormal liver tests”, and to two of the references. The journal apologises for the errors.

In the abstract, under methods, the second sentence should read “Patients with abnormal liver tests were assessed and followed throughout and after pregnancy. Medical advice was provided to obstetric teams.”

Pregnancy specific conditions

Pre-eclampsia was a common underlying abnormality seen in 68 patients (48%) but was the sole identifiable cause of liver dysfunction in only 15. Complete HELLP and incomplete HELLP syndrome occurred in 30 patients of whom 29 were pre-eclamptic making this the commonest diagnosis accounting for abnormal liver tests. In most of those patients with incomplete or partial HELLP syndrome, the platelet count dropped abruptly by more than 50% in parallel with abnormal liver tests. OC was diagnosed in 23 patients (16%); two of whom had pre-eclampsia. Eleven patients (8%) had deranged LFT associated with hyperemesis gravidarum and all responded well to conservative management or oral steroids; none of these developed pre-eclamptic liver dysfunction later in pregnancy. AFLP was diagnosed in five patients (4%), three of whom needed prolonged post-natal hospitalisation because of multi-organ involvement; none of these five patients had pre-eclampsia. Clinical details of these patients have been presented and published recently.

In “Other contributory conditions” the first sentence should read “Seventeen patients had abnormal liver tests in association with sepsis,

Table 1 Aspartate aminotransferase (AST), gamma glutamyl transpeptidase (γGT), and bilirubin values for specific pregnancy related liver disorders

<table>
<thead>
<tr>
<th></th>
<th>AST (U/l)</th>
<th>γGT (U/l)</th>
<th>Bilirubin (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HELLP syndrome</td>
<td>66 (41–123)</td>
<td>24 (6–209)</td>
<td>13 (4–15)</td>
</tr>
<tr>
<td>Obstetric cholestasis</td>
<td>210 (30–519)</td>
<td>29 (8–278)</td>
<td>14 (6–34)</td>
</tr>
<tr>
<td>Pre-eclamptic liver dysfunction alone</td>
<td>68 (36–210)</td>
<td>18 (7–51)</td>
<td>7 (3–12)</td>
</tr>
<tr>
<td>Hyperemesis gravidarum</td>
<td>51 (9–280)</td>
<td>23 (2–64)</td>
<td>25 (4–33)</td>
</tr>
<tr>
<td>AFLP</td>
<td>278 (86–542)</td>
<td>50 (22–209)</td>
<td>50 (19–61)</td>
</tr>
</tbody>
</table>

Values are median (range). HELLP, haemolysis, elevated liver enzymes, low platelets; AFLP, acute fatty liver of pregnancy.
most commonly caused by urinary tract infection (9 patients); 5 of these 17 were also pre-eclamptic."

References 11 and 12 should be as follows:

Authors affiliations are:
C L Ch'ng, J G C Kingham, Department of Gastroenterology, Singleton Hospital, Swansea, UK
M Morgan, Department of Obstetrics and Gynaecology, Singleton Hospital, Swansea, UK
I Hainsworth, Department of Clinical Pathology, Morriston Hospital, Swansea, UK

NOTICES

The national register of hepatitis C infections with a known date of acquisition.

The register steering group invite clinical and epidemiological researchers to submit proposals to access data held in the register. It is envisaged that a variety of studies might benefit from linkage with or access to the register, and proposals from all specialties and institutions are welcome. Any researchers interested in applying for access to information held within the national register should contact the register co-ordinator (see below) for a list of available data and an application form. Study proposals should then be submitted to the register co-ordinator by 16 December 2002.

Table 2 Diagnoses accounting for abnormal liver tests

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Pregnancy specific</th>
<th>n</th>
<th>No with ↑ AST</th>
<th>No with ↑ γGT</th>
<th>No with ↑ bilirubin</th>
<th>No with ↑ urate</th>
<th>No with low platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>HELLP syndrome</td>
<td>30</td>
<td>30</td>
<td>12</td>
<td>10</td>
<td>29</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Obstetric cholestasis</td>
<td>23</td>
<td>22</td>
<td>10</td>
<td>2</td>
<td>17</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pre-eclampsia alone</td>
<td>15</td>
<td>14</td>
<td>4</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hyperemesis gravidarum</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Acute fatty liver of pregnancy</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hepatic infarct/haematoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Group 2—Other conditions

| Postoperative (caesarean section) | 22 | 21 | 10 | 3 | 21 | 7 |
| Sepsis | 17 | 17 | 6 | 2 | 12 | 2 |
| Placental pathologies | 12 | 11 | 4 | 3 | 9 | 3 |
| Diabetes | 8 | 6 | 5 | 1 | 5 | 4 |
| Drug related | 4 | 1 | 4 | 1 | N/A | 2 |
| Bile duct stones | 3 | 3 | 2 | 2 | 1 | 0 |
| Hepatitis C | 2 | 2 | 1 | 0 | 0 | 0 |

Group 3—Diagnosis obscure | 14 | 8 | 4 | 1 | 6 | 0 |

N/A, not available.

Further information: Dr Helen Harris (Registrar Co-ordinator) or Ms Lisa Beck (Research Assistant), Immunisation Division, Communicable Diseases Surveillance Centre, Public Health Laboratory Service, 61 Colindale Avenue, London NW9 6EQ, Tel: +44 (0)20 8200 6868 ext 4496; fax: +44 (0)20 8200 7868; email: hharris@phls.nhs.uk or lbeck@phls.nhs.uk

Table 3 Timing of liver dysfunction: Onset of specific pregnancy related liver disorders (in weeks’ gestation)

| HELLP syndrome | 36 (25–38) |
| Obstetric cholestasis | 35 (21–39) |
| Preeclamptic liver dysfunction | 37 (25–40) |
| Hyperemesis gravidarum with liver dysfunction | 9 (6–14) |
| AFLP | 38 (32–38) |

Values are median (range).

HELLP, haemolysis, elevated liver enzymes, low platelets; AFLP, acute fatty liver of pregnancy.

17th International Workshop on Therapeutic Endoscopy

This will be held on 3–5 December 2002 in Hong Kong. Further information: Professor SC Sydney Chung, Endoscopy Centre, Prince of Wales Hospital, Shatin, NT, Hong Kong. Tel: +852 2632 2233; fax: +852 2635 0075; email: info@hksde.org

Advances in the Inflammatory Bowel Diseases

This conference will take place on 6–7 December 2002 in New York, USA. Further information: Heather Drew, Imedex, 70 Technology Drive, Alpharetta, GA 30005-3969, USA. Tel: +1 770 751 7332; fax: +1 770 751 7334; email: h.drew@imedex.com; website: www.imedex.com

The Future of Gastro-entero-hepato-pancreatology is bright

This Academic Farewell Symposium of Guido NJ Ytgaat will be held on 12 December 2002 in Amsterdam, the Netherlands. Deadline for registration is 1 November 2002 (no registration fee) and registration should be done via email to j.goedkop@amc.uva.nl.

Cancer of Oesophagus and Gastric Cardia: from Gene to Cure

This conference will be held on 13–15 December 2002 in Amsterdam, The Netherlands. Further information: European Cancer Centre, PO Box 9236, NL 1006 AE Amsterdam, The Netherlands. Tel: +31 (0)20 346 2547; fax: +31 (0)20 346 2525; email: ecc@lkca.nl

Imaging of the Abdomen: an Update

This will be held on 23–24 January 2003 in Amsterdam, the Netherlands. Further information: visit the website www.epgs.nl or email epgs@amc.uva.nl. Tel: +31 20 66 3926/4386.

Surgery of the Foregut

This meeting will be held on 17–18 February 2003 in Florida, USA. Further information: Cleveland Clinic Florida, Office of CME, 2950 Cleveland Clinic Boulevard, Weston, FL 33331, USA. Tel: +1 954 659 5490; (toll free): +1 866 293 7866; fax: +1 954 659 5491; email: cme@ccf.org