Human transforming growth factor α (TGF-α) 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 α (TGF-α) 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-α 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-α1–50 is cleaved to TGF-α1–43 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-α1–43. TGF-α1–43 had only half of the ability to maximally stimulate [3H]thymidine incorporation into primary rat hepatocytes (28 177 (1130) DPM/well for 2.16 nM TGF-α1–50 v 63 184 (3536) DPM/well for TGF-α1–43; p<0.001). A similar reduced potency was seen when used in an indomethacin induced rat gastric damage model (0.18 µmol/kg/h of TGF-α1–43 reduced ulcer area by 19% whereas TGF-α1–50 reduced area by 62%; p<0.001).

Conclusions: TGF-α1–43 is cleaved to the TGF-α1–43 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.

MATERIAL AND METHODS

Materials

Recombinant and purified native human TGF-α 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.

Methods of analyses of samples to determine the forms of TGF-α

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 octadeцилсилан (C18) cartridge chromatography, as described by Elson and colleagues. 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, Rhodyne-7725 sample injector, and a 1 ml injection loop. The column used was an analytical “Jupiter” C5, 300 A, 3 µm (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-10 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.

Abbreviations: HPLC, high pressure liquid chromatography; AcN, acetonitrile; TFA, trifluoroacetic acid; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; PPI, proton pump inhibitor; RIA, radioimmunoassay; TGF-α, transforming growth factor α.
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/tube of donkey antisaep 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 (T_total) 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 log concentration by least squares. These showed a small but signiﬁcant decrease in the immunoreactivity of TGF-α which had been treated with 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, pH 12.4). Mass spectroscopy without further separation. The other three 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

Assay of biological activity of different forms of TGF-α in vitro
The ability of TGF-α to prevent gastric damage by indomethacin and restraint in rats was assessed using previously validated methods. Under light ether anaesthesia, rats (male Sprague Dawley, 225–250 g) had two subcutaneous cannula inserted into the back of the neck and were then placed in Bullman restraint cages. Once the animals had recovered, 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 inltered with 4 ml of 10% formalin. The next day they 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 (mm²/10 cm²) 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. 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 assayed 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

Methods of analyses of biological activity
In vitro assay
Background to method
Primary rat hepatocytes provide a robust reproducible method for evaluating 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. 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. 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. 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).

To prevent gastric damage by indomethacin and restraint in rats was assessed using previously validated methods. Under light ether anaesthesia, rats (male Sprague Dawley, 225–250 g) had two subcutaneous cannula 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 inltered with 4 ml of 10% formalin. The next day they 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 (mm²/10 cm²) 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. 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.

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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 Nα-benzoyl-DL-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°C prior to HPLC and RIA.

Study 3: changes in the form of intragastric TGF-α 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°C prior to HPLC and RIA.

Study 4: biological effects of changes in the form of TGF-α

Preparation of TGF-α

To determine the effect of acid pepsin digestion on the biological activity of TGF-α, four aliquots of 100 µg of previously pooled recombinant TGF-α 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-α) or 1 ml of Tris buffer pH 7.0 containing 1 mg pepsin (that would not be expected to digest TGF-α). 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-α (shown) or HCl alone (not shown) eluted as a single peak in fraction 11 (A). TGF-α that had been preincubated in acid and pepsin for 10 minutes eluted as two peaks equating to a mixture of TGF-α (fractions 11 and 12) and TGF-α (shown) or HCl alone (not shown) eluted as a single peak in fraction 14 (A). TGF-α which had been incubated in HCl without pepsin, and Tris pepsin treated TGF-α, were frozen and stored at −20°C prior to HPLC and RIA.

In vitro assay

Various concentrations (0–9 nM) of either intact TGF-α (Tris/ pepsin treated) or the acid pepsin treated TGF-α were added to the hepatocytes and thymidine incorporation determined 26 hours later. For both intact and acid pepsin treated TGF-α, each dose of TGF-α 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-α (Tris-pepsin treated TGF-α) at either 0.18 or 0.90 µmol/kg/h, or acid pepsin treated TGF-α at either 0.18 or 0.90 µmol/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-α in acid pepsin in vitro

Intact recombinant TGF-α, purified TGF-α, TGF-α which had been incubated in HCl without pepsin, and Tris pepsin treated TGF-α gave a single peak on mass spectroscopy corresponding to intact TGF-α (B). Measured mass was always in the range 5546–5554 (expected molecular weight of TGF-α 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-α.
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-α1–43 (measured molecular weight 4796, theoretical molecular weight 4796). HPLC separation followed by RIA showed one peak of immunoreactivity in fractions 11 and 12 corresponding to TGF-α1–50 [A]. Changing the intragastric infusate to sodium bicarbonate caused the pH to rise to approximately 7 and the predominant form of TGF-α remained intact (fig 1B).

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-α1–43 form. TGF-α incubated in acid alone resulted in a peak in fraction 14 indicating intact TGF-α1–50 (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) give one peak corresponding to TGF-α1–50, 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-α1–43. 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-α1–43, and TGF-α1–50, 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-α1–50 (fig 2A).

During bicarbonate perfusion, the pH of the samples were all in the range 6.8–7.2 and the peak of TGF-α1–50 corresponded to TGF-α1–43 with a slight shoulder in the position of TGF-α1–43 (fig 2B). The subject who had saline perfusion throughout showed no change in the form of TGF-α present during the final period (TGF-α1–50).

**Study 4: biological activity assays**

[3H]Thymidine incorporation into primary rat hepatocytes Both forms of TGF-α stimulated thymidine uptake in hepatocytes in a dose dependent manner (fig 3). However, maximal

**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-α1–50 [A]. Changing the intragastric infusate to sodium bicarbonate caused the pH to rise to approximately 7 and the predominant form of TGF-α in the juice to be the 1–50 form (fraction 14) [B].
stimulation by TGF-α(1–43) was approximately double that caused by TGF-α(1–36) (76447 (5654) vs 35375 (2710) DPM/well for 9 nM). For all doses studied, biological activity was greater for the TGF-α(1–36) form compared with the same dose of TGF-α(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 ($F_{2,29}=13.238$, $p=0.0000$) and form ($F_{1,29}=20.597$, $p=0.000$) of TGF-α. For both doses tested, TGF-α(1–43) was significantly more potent than the TGF-α(1–36) form in its ability to reduce injury ($p<0.01$, see fig 4).

Assessment using the microscopic 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 µmol/kg/h of the TGF-α(1–36) was slightly higher than that seen in animals receiving 0.18 µmol/kg/h of TGF-α(1–43), suggesting that, in this particular model, the intact form is about 3–5 times as potent as TGF-α(1–43).

**DISCUSSION**

We have shown that TGF-α is susceptible to cleavage by acid peptic digestion in vivo and in vitro. Under normal basal acidic conditions, the predominant form in gastric juice is TGF-α(1–36) 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(1–43)) form. We have also shown that the acid digested TGF-α(1–43) has about half to one fifth of the biological activity of the intact TGF-α(1–43) 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 colonic (for example, HT29) or gastric (for example, AGS) origin have previously been used by us and other groups to assess proliferation of cell lines in vitro, and are probably sufficient to stimulate proliferation of human gastric explants. In addition, pathophysiological relevance for this concentration of TGF-α in gastric juice is supported by the finding that saliadenectomy 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 increased susceptibility to noxious agents with increased ulceration and delayed healing. 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 immunoreactive. 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. 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 µmol/kg/h. The lower dose of TGF-α probably did not affect gastric acid secretion but the higher dose may have done so because 1.8
μmol/kg/h of TGF-α, administered intravenously, decreases gastric acid secretion by 72%. This may be relevant to the mechanism by which TGF-α decreased gastric damage in our rat model as we have previously shown the damage to be acid dependent. 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, 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 colleagues examined which areas of the TGF-α molecule played a key role in its interaction with the erbB-1 receptors. 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-α-C having only 1/1000 of the activity of TGF-α. This is a much lower level 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-C, 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.

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 phenotype, whereas multiple knockouts or deletion of the EGF-R itself resulting in profound effects on the gut. The current series of studies suggest that changing the size of the TGF-α molecule from the truncated form to TGF-α-C 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. 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. Further studies appear warranted.

References

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 minimise 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 melaena with an association of airways disease, left ventricular failure (including radiographically validated left ventricular failure) and chronic obstructive airways disease.

The authors of Marchbank et al (J R Soc Med 1998;91:518–23) disagreed with 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 minimise of surgical intervention and, as shown below, postoperative management at the intensive care level, may be more crucial to survival than comorbidity as such.

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Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>AST (U/l)</th>
<th>γGT (U/l)</th>
<th>Bilirubin (µmol/l)</th>
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<tbody>
<tr>
<td>HELLP syndrome</td>
<td>66 (41–423)</td>
<td>24 (6–209)</td>
<td>13 (4–155)</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:


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 welcomed. 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—Pregnancy specific</th>
<th>n</th>
<th>Na with ↑ AST</th>
<th>Na with ↑ γGT</th>
<th>Na with ↑ bilirubin</th>
<th>Na with ↑ urate</th>
<th>Na with low platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>HELLP syndrome (5 complete, 25 partial)</td>
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<td>30</td>
<td>12</td>
<td>10</td>
<td>29</td>
<td>30</td>
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<tr>
<td>Obstetric cholestasis</td>
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<td>22</td>
<td>10</td>
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<td>0</td>
</tr>
<tr>
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<td>11</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Acute fatty liver of pregnancy</td>
<td>5</td>
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<td>3</td>
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<td>3</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>
</tr>
</tbody>
</table>

Group 2—Other conditions

Postpartum (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 (Register 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

The 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-pancreatologie is bright

This Academic Farewell Symposium of Guido NJ Ytgent 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 2523; email: epgs@amc.uva.nl

Imaging of the Abdomen: an Update

This will be held on 23–24 January 2003 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 2523; email: epgs@amc.uva.nl

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 3331, USA. Tel: +1 954 659 5490; (toll free: +1 866 293 7866); fax: +1 954 659 5491; email: cme@ccf.org