Hepatocellular integrity during and after isoflurane and halothane anaesthesia in surgical patients

P. Tiainen and P. H. Rosenberg

Summary

Subclinical disturbance in hepatocellular integrity, indicated by glutathione transferase Alpha (GSTA), has been associated with halothane, sevoflurane and propofol, but not with isoflurane anaesthesia. We anaesthetized 82 patients with isoflurane or halothane at 1 MAC for superficial surgery. GSTA concentration were measured with a sensitive time-resolved immunofluorometric assay in serum samples. GSTA concentrations increased from a baseline value of geometric mean 1.8 µg litre⁻¹ (95% confidence intervals 1.4–2.2 µg litre⁻¹) to a peak of 4.3 (3.3–5.7) µg litre⁻¹ in the isoflurane group and from 2.1 (1.6–2.9) µg litre⁻¹ to 6.2 (4.1–9.5) µg litre⁻¹ in the halothane group. The change in GSTA was significant within groups but the difference between groups was not significant. Two patients exhibited an unexpectedly large increase in GSTA (peaks 370 and 620 µg litre⁻¹) and a mild increase in alanine aminotransferase after halothane anaesthesia. We conclude that hepatocellular integrity was mildly disturbed after isoflurane and halothane anaesthesia but there was no difference between anaesthetics. Halothane anaesthesia may be associated with more advanced hepatocellular disturbance in some cases. (Br. J. Anaesth. 1996; 77: 744–747)

Key words


Disturbance in hepatocellular integrity indicated by a sensitive marker, glutathione transferase Alpha (GSTA), has been noted after halothane, sevoflurane and propofol anaesthesia, but not after isoflurane anaesthesia. There is some disagreement between studies which used an enzyme immunoassay or a time-resolved immunofluorometric assay for GSTA measurements after sevoflurane and propofol anaesthesia. For example, the enzyme immunoassay kit does not appear to be sensitive enough to detect small changes in serum concentrations of GSTA. In addition, because of the relatively short half-life of GSTA in serum, GSTA should be assayed in samples collected at short intervals. This has not been the case in several recently published studies involving anaesthesia and GSTA where no samples were obtained for several hours after the end of anaesthesia. Therefore, we have attempted to confirm the reported differential influence of isoflurane and halothane anaesthesia on hepatocellular integrity by using a new, sensitive, time-resolved immunofluorometric assay for GSTA and short sampling intervals.

Patients and methods

The study was approved by the Ethics Committee of the Surgical Hospital, Helsinki University Central Hospital. Informed consent was obtained from all patients. We studied 82 patients, aged 22–64 yr, with a body mass index (BMI) < 30 kg m⁻² (weight × height⁻²) undergoing superficial surgery during general anaesthesia lasting at least 2 h (table 1). Exclusion criteria were patients with known liver disease or a history of heavy alcohol consumption, those with hyperthyroidism or receiving thyroxine medication, those receiving medication likely to affect the liver, and those who were suspected to have had an adverse anaesthesia-related effect after a previous inhalation anaesthetic. Administration of paracetamol or corticosteroids was not allowed within 3 days before or during the study.

Patients were allocated randomly to one of two groups of 41 patients each, to receive either isoflurane or halothane anaesthesia. Premedication comprised oral diazepam, approximately 0.2 mg kg⁻¹. Glycopyrroponium 0.2 mg, fentanyl 3 µg kg⁻¹, thiopentone 3–6 mg kg⁻¹ and vecuronium 0.1 mg kg⁻¹ were given during induction of anaesthesia. After tracheal intubation, normocapnia was maintained with mechanical ventilation using oxygen-enriched air (P\textsubscript{FIO\textsubscript{2}} = 0.35) without positive end-expiratory pressure. Anaesthesia was maintained with isoflurane at an expiratory concentration of 0.8%, which was 0.68 (SD 0.04) MAC (age-corrected mean) or 0.5% halothane, which was 0.65 (0.03) MAC, for 30 min. Thereafter, isoflurane was maintained at 1.2%

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(1.02 (0.05) MAC) or 0.75% halothane (0.98 (0.04) MAC), until the end of anaesthesia. Small increments of fentanyl 0.05–0.1 mg were given if heart rate or arterial pressure increased. Nitrous oxide ($F_{NO_2} = 0.65$) was added if a patient expressed clinical signs of inadequate anaesthesia after fentanyl increments. If neuromuscular block was needed, it was maintained with increments of vecuronium 1 mg and antagonized with neostigmine 2 mg and glycopyrronium 0.4 mg. Inspiratory and expiratory concentrations of isoflurane and halothane were monitored continuously (Capnomac Ultima, Datex, Helsinki, Finland). Other monitoring included non-invasive arterial pressure every 5 min and continuous electrocardiography, pulse oximetry, and inspiratory and expiratory $P_O_2$ and $P_CO_2$ (Capnomac Ultima and Cardiopac, Datex, Helsinki, Finland). Haemodynamic state was characterized by calculating the mean of systolic arterial pressure and heart rate during anaesthesia. The threshold of hypotension was defined as systolic arterial pressure 85 mm Hg, and the degree of hypotension was calculated as the area under the curve (AUC) of hypotension. Hypotension was defined as an AUC value of >100 mm Hg min. Hypotension was managed by increasing the rate of fluid administration and if nitrous oxide was administered, it was discontinued (two patients in each group). After anaesthesia, patients were observed for 3 h in the post-anaesthesia care unit and thereafter on the ward. After operation, oxycodone 0.07 mg kg$^{-1}$ i.v. or 0.14 mg kg$^{-1}$ i.m. was given when a patient requested pain medication.

**SAMPLES AND LABORATORY ANALYSIS**

Peripheral venous blood was obtained before anaesthesia (baseline), hourly during anaesthesia, and 0, 1, 3, 6 and 24 h after the end of anaesthesia. Serum was stored at $-20$ °C, that is at a temperature which we have found to conserve the antigenic integrity of GSTA for an immunoassay.

GSTA concentrations were measured with a time-resolved immunofluorometric assay according to a standard assay procedure, except that the Eu$^{3+}$ label was incubated for 3 h. Samples from each patient were analysed in one assay cycle. The detection limit of the assay was 0.03 µg litre$^{-1}$ and the measuring range was 0.3–1000 µg litre$^{-1}$ at 10-fold serum dilution. Mean intra-assay and inter-assay coefficients of variation (cv) were 4.5–5.4% and 11.3–17.0%, respectively. The reference ranges of GSTA were 0.7–6.0 µg litre$^{-1}$ and 0.7–14 µg litre$^{-1}$ for females and males, respectively.

Baseline concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transferase and bilirubin were measured in routine hospital laboratory tests in serum samples obtained before anaesthesia. The detection limits for AST and ALT were less than 5 iu litre$^{-1}$ and the inter-assay cv was 2.2–3.6%. ALT and AST were also measured 24 h after the end of anaesthesia.

**STATISTICAL ANALYSIS**

The skewed distributions of GSTA concentrations and aminotransferase activities were normalized by logarithmic transformation. Changes were compared between groups and at successive times using repeated measures analysis of variance (ANOVA). Student’s t test was used for comparison of the peak and area under the concentration curve (AUC) values between the groups on a logarithmic scale. Systat software was used in computing. For power analysis, GSTA concentration >66% above or >40% below the reference was considered as clinically significant, that is 0.22 on log$_{10}$ scale. The sd values of GSTA concentrations were predicted to be 0.3 on log$_{10}$ scale. Forty-one patients in both groups were required for a power of 90% at $\alpha = 0.05$ or 36 patients for 85%, respectively.

**Results**

Results from 75 patients were analysed (38 in the isoflurane group and 37 in the halothane group). Six patients were excluded because the baseline GSTA concentration was higher than the upper limit of the reference range, and one patient because she underwent re-operation because of bleeding. Patients in the isoflurane and halothane groups were comparable in sex distribution, age, height, weight, BMI, type of surgery and incidence of malignancy (table 1). Peroperative bleeding was 500 ml or less, except in four patients in the isoflurane group (600–1100 ml) and in five patients in the halothane group (600–1600 ml). Duration of anaesthesia and need for fentanyl, nitrous oxide, vecuronium and neostigmine were similar in both groups (table 2). Mean values for arterial pressure were the same in both tables 1 and 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics and type of surgery (mean (sd or range) or number). Body mass index (BMI) = weight $\times$ height$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Isoflurane</strong> ($n=38$)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
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</tr>
<tr>
<td>Age (yr)</td>
<td>41 (22–64)</td>
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<tr>
<td>Height (cm)</td>
<td>169 (8.7)</td>
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<tr>
<td>Weight (kg)</td>
<td>66 (10.5)</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>23.2 (2.3)</td>
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<tr>
<td>Type of surgery</td>
<td></td>
</tr>
<tr>
<td>Mammary gland</td>
<td>19</td>
</tr>
<tr>
<td>Thyroid gland</td>
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<tr>
<td>Male genital</td>
<td>8</td>
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<tr>
<td>Malignancy</td>
<td></td>
</tr>
<tr>
<td>Mammary gland</td>
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<td>Thyroid gland</td>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Anaesthesia characteristics (mean (sd) or number). $\ast$($P&lt;0.05$) between groups</th>
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<tr>
<td></td>
<td><strong>Isoflurane</strong> ($n=38$)</td>
</tr>
<tr>
<td>Duration of anesthesia (min)</td>
<td>190 (70)</td>
</tr>
<tr>
<td>Thiopentone (mg)</td>
<td>349 (99)</td>
</tr>
<tr>
<td>Fentanyl (mg)</td>
<td>0.38 (0.13)</td>
</tr>
<tr>
<td>Vecuronium (mg)</td>
<td>11.9 (4.1)</td>
</tr>
<tr>
<td>Nitrous oxide (No. of patients)</td>
<td>6</td>
</tr>
<tr>
<td>Neostigmine (No. of patients)</td>
<td>16</td>
</tr>
<tr>
<td>Haemodynamics during anaesthesia</td>
<td></td>
</tr>
<tr>
<td>Mean systolic arterial pressure (mm Hg)</td>
<td>100.8 (6.4)</td>
</tr>
<tr>
<td>Mean heart rate (beat min$^{-1}$)</td>
<td>71.3 (10.1)$\ast$</td>
</tr>
<tr>
<td>Hypotensive patients ($\ast$)</td>
<td>7</td>
</tr>
</tbody>
</table>
groups, but heart rate was higher in the isoflurane group (table 2).

Seven patients in the isoflurane group and eight patients in the halothane group were hypotensive during anaesthesia. AUC values of hypotension ranged from 110 to 790 mm Hg min (median 290 mm Hg min) in the isoflurane group and from 100 to 450 mm Hg min (median 220 mm Hg min) in the halothane group.

GSTA concentrations varied in the isoflurane and halothane groups at successive times (\( P < 0.001 \), repeated measures ANOVA). GSTA concentrations were significantly (\( P < 0.01 \)) higher than baseline from 2 h after induction to 3 h after the end of anaesthesia in both groups. In the isoflurane group, GSTA concentration was less than baseline at 6 and 24 h after anaesthesia. One patient exhibited an unexplained secondary increase in GSTA from 1.4 to 110 mm Hg min (median 290 mm Hg min) in the halothane group.

There were no significant differences in GSTA concentrations between the isoflurane and halothane groups during the study. Neither the peak nor AUC of GSTA differed significantly between the isoflurane and halothane groups (table 3). There were nine patients in the isoflurane group and 12 patients in the halothane group with peak GSTA values above the reference range during or immediately after anaesthesia. One patient exhibited an unexplained secondary increase in GSTA from 1.4 to 19.1 µg litre\(^{-1}\) at 24 h after isoflurane anaesthesia.

The peak concentration of GSTA (geometric mean) was 4.7 (95% confidence interval 3.4–6.6) µg litre\(^{-1}\) in hypotensive patients and 5.3 (3.9–7.2) µg litre\(^{-1}\) in normotensive patients (ns). The corresponding results for AUC were 5.3 (2.4–11.8) µg litre\(^{-1}\) h and 7.4 (4.8–11.5) µg litre\(^{-1}\) h (ns), respectively. The increase in GSTA was not associated with the addition of nitrous oxide, duration of anaesthesia (<180 min vs ≥180 min), type of surgery, malignancy, peroperative bleeding (<500 ml vs ≥500 ml), age (<40 yr vs ≥40 yr), sex or BMI (<23 kg m\(^{-2}\) vs ≥23 kg m\(^{-2}\)) when the peak and AUC values of GSTA were compared by \( t \) test and the successive GSTA concentrations with repeated measures ANOVA with one grouping factor (each of those mentioned above) or two grouping factors (volatile anaesthetic and each of those mentioned above).

Two patients (aged 46 and 53 yr; BMI 27.0 and 27.3 kg m\(^{-2}\)) exhibited an unexpectedly large increase in GSTA during and after halothane anaesthesia for surgery of breast cancer (fig. 1). There was a rapid decrease in GSTA to the reference range after anaesthesia in these two patients. No other signs or symptoms except for an increase in ALT (from 25 to 50 iu litre\(^{-1}\) and from 17 to 121 iu litre\(^{-1}\)) were observed and no obvious reason for the increase in GSTA was evident. Both patients recovered normally and ALT, AST, glutamyl transferase, bilirubin and GSTA concentrations were within reference ranges in control samples approximately 1 yr later.

There was a minor decrease in aminotransferase activities (table 4), but no significant difference between groups. There were no clinical signs or symptoms of hepatic disorder in any patient.

### Discussion

We found a small, short-lasting increase in GSTA concentrations in serum after isoflurane and halothane anaesthesia. This indicated a mild subclinical disturbance in hepatocellular integrity.\(^1\) The mechanisms of the hepatic effect are unclear. Hypotension, nitrous oxide, duration of anaesthesia, type of surgery, malignancy, bleeding, age, sex or BMI were not associated with the increase in GSTA. An unexplained secondary increase in GSTA without significant primary peak in the isoflurane group was similar to that described in previous studies.\(^1, 2\)

The lack of difference in GSTA between isoflurane and halothane contrasts with the results of earlier studies.\(^1, 2\) Compared with the study of

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**Table 3** GSTA concentrations (µg litre\(^{-1}\)) in serum and area under the concentration curve (AUC) values (geometric mean (95% confidence interval)). Significant differences compared with baseline: **\( P < 0.01 \), ***\( P < 0.001 \).  

<table>
<thead>
<tr>
<th></th>
<th>Isoflurane (n = 38)</th>
<th>Halothane (n = 37)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 h after induction</td>
</tr>
<tr>
<td></td>
<td>1.8 (1.4–2.2)</td>
<td>1.9 (1.4–2.3)</td>
</tr>
<tr>
<td></td>
<td>2.1 (1.6–2.9)</td>
<td>2.3 (1.7–3.0)</td>
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<tr>
<td></td>
<td>17.7 (15.8–19.9)</td>
<td>17.7 (15.5–20.3)</td>
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</tbody>
</table>

**Table 4** Aminotransferase activities (iu litre\(^{-1}\)) in serum (geometric mean (95% confidence interval)). **\( P < 0.01 \) compared with baseline.  

<table>
<thead>
<tr>
<th></th>
<th>Isoflurane (n = 38)</th>
<th>Halothane (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT baseline</td>
<td>14.9 (13.0–17.5)</td>
<td>17.4 (15.0–20.3)</td>
</tr>
<tr>
<td>24 h after anaesthesia</td>
<td>12.6 (11.0–14.6)**</td>
<td>15.2 (12.0–19.1)</td>
</tr>
<tr>
<td>AST baseline</td>
<td>18.1 (16.2–20.2)</td>
<td>20.0 (18.3–22.0)</td>
</tr>
<tr>
<td>24 h after anaesthesia</td>
<td>17.7 (15.8–19.9)</td>
<td>17.7 (15.5–20.3)**</td>
</tr>
</tbody>
</table>

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**Figure 1** GSTA concentrations in serum. Lines indicate GSTA concentrations in two patients who exhibited an unexpectedly large increase in GSTA after halothane anaesthesia. Hatched area contains all GSTA concentrations in the other 73 patients, including a secondary increase in one patient 24 h after isoflurane anaesthesia.
Murray, Rowlands and Trinick, we exposed younger patients (mean age 41 vs 59 yr) to lower concentrations of inhalation anaesthetics (1.0 vs 2.0 MAC) for shorter intervals (mean duration 3.2 vs 10 h). In their study, aspartate aminotransferase activity increased to 110 iu litre$^{-1}$ (mean 48 h after anaesthesia, which suggests more advanced disturbance in hepatocellular integrity than in studies without any increase in aminotransferases. The relatively insensitive enzyme immunoassay for GSTA used by Murray, Rowlands and Trinick, and infrequent sampling after anaesthesia may be the reason why there was no change in GSTA concentration after isoflurane anaesthesia.

The discrepancy between our study and that of Hussey and colleagues is unclear. Patients with recent exposure to halothane (within 6 months) were not included in either study but differences in earlier exposure to halothane could be a confounding factor. We speculate that differences in the concentrations of inhalation anaesthetics and ventilation mode may explain why Hussey and colleagues obtained higher GSTA concentrations after halothane than after isoflurane anaesthesia. The concentration of volatile anaesthetic was standardized (about 1.0 MAC) in our study. Ventilation was controlled mechanically and normoventilation was achieved by monitoring end-tidal $P_CO_2$. In contrast, in the study of Hussey and colleagues, the inspiratory concentration of volatile anaesthetic varied (1.0–3.0% isoflurane and 1.0–1.5% halothane in oxygen–nitrous oxide) and ventilation was spontaneous.

There is substantial evidence that immunological mechanisms may cause delayed hepatic injury after halothane anaesthesia (halothane hepatitis). However, the time course of the changes in GSTA in our study did not indicate immunological mechanisms. Very deep anaesthesia is deleterious to the hepatic circulation. This effect is more pronounced with halothane than with other anaesthetics, but the differences are small at 1 MAC concentration. The direct toxicity of halothane on hepatocytes under hypoxic conditions or glutathione depletion could also cause liver injury, but in a recent well-controlled study the authors rejected these mechanisms.

It would be tempting to implicate halothane as the cause of the unexpectedly large increase in GSTA in two of our patients after halothane anaesthesia. However, we found no mechanism to explain the relatively large changes in GSTA. Neither of these patients used any medication nor had any other known disease except breast cancer. They were not hypotensive during anaesthesia. Both had been anaesthetized twice, 1–22 yr before the present anaesthetic. A volatile anaesthetic (isoflurane) had been administered in one of the previous anaesthetics.

By using a sensitive immunoassay for measurement of serum concentrations of GSTA, we have previously detected small increases in GSTA after halothane, sevoflurane and propofol anaesthesia. This study showed that hepatocellular integrity appeared to be disturbed to a similar degree during general anaesthesia with isoflurane or halothane at 1.0 MAC concentration. However, in two patients halothane anaesthesia was associated with large increases in serum concentrations of GSTA with minor increases in ALT. The significance of this is not clear.

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References