Purine nucleoside use as surrogate markers of cerebral ischaemia during local and general anaesthetic carotid endarterectomy

Owain Fisher1,2, Ruth A Benson1,3, Faming Tian2,4, Nicholas E Dale2,4 and Christopher HE Imray1,2

Abstract

Objectives: In periods of cerebral ischaemia, adenosine triphosphate is metabolised, leading to accumulation of adenosine inosine and hypoxanthine. These can be measured in real time using peripheral blood samples intraoperatively. The primary aim of this study was to describe changes in purine concentrations in a cohort of patients undergoing carotid endarterectomy under general anaesthetic, and to evaluate correlation between changes in values with major perioperative steps. The secondary aim was to compare changes in concentrations with a previous cohort of patients who had undergone carotid endarterectomy under local anaesthetic.

Methods: This was a prospective observational study. Purine concentrations were determined from arterial line samples and measured via an amperometric biosensor at specific time points during carotid endarterectomy. Mean arterial pressure was manipulated to maintain steady cerebral perfusion pressure throughout the procedure. These results were analysed against data from a cohort of patients who underwent carotid endarterectomy under local anaesthetic in previously published work.

Results: Valid results were obtained for 37 patients. Purine concentrations at baseline were 3.02 ± 1.11 µM and 3.16 ± 1.85 µM for the unshunted and shunted cohorts, respectively. There was no significant change after 30 min of carotid clamping at 2.07 ± 0.89 and 2.4 ± 3.09 µM, respectively (both p > 0.05). Peak purine during the clamp phase in the loco-regional anaesthetic cohort was 6.70 ± 3.4 µM, which was significantly raised compared to both general anaesthetic cohorts (p = 0.004). There were no perioperative neurological events in either cohort.

Conclusion: This small study does not demonstrate conclusive evidence that purine nucleosides can be used as a marker of cerebral ischaemia; the comparisons to the loco-regional anaesthetic data offer information about differences in the cerebral adenosine triphosphate metabolism between general anaesthetic and loco-regional anaesthetic. We hypothesise that the lack of a rise in purine nucleosides under general anaesthetic may be caused by a decrease in the cerebral metabolic rate and loss of metabolic rate-blood flow coupling caused by general anaesthetic agents.

Keywords

Biomarker, hypoperfusion, shunt, stroke

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Introduction

Adenosine triphosphate (ATP) is essential for cellular function.1 Under normal physiological conditions, ATP is continuously produced by the oxidative phosphorylation of adenosine diphosphate (ADP). ATP is then metabolised to release the energy necessary for cellular functioning.2 During periods of tissue ischaemia or hypoxia, alternative pathways are needed. These pathways allow metabolism of ATP and ADP by enzymes (AMP deaminase and 5′ nucleosidase) that
are otherwise dormant. This leads to the production of intermediate purine nucleosides, namely adenosine, inosine and hypoxanthine. Accumulation of these intermediate purines occurs as further metabolism to xanthine, urate and uric acid is oxygen dependent and therefore also inhibited. These nucleosides are released into the extracellular matrix and are ultimately detectable in the peripheral bloodstream.

Purine release is highly sensitive for ischaemia. The published literature has identified purine release in cerebral and myocardial ischaemia as well as during hypoxia, induced by simulating high altitude. Historically, their clinical application had been limited by invasive testing methods and biochemical instability of purines resulting in rapid metabolism and reuptake causing poor reproducibility of measurements with older analysis techniques. Recently, our group reported the results of a novel amperometric sol–gel biosensor (SMARTchip, Sarissa Biomedical, Warwick, UK) to instantaneously measure changes in nucleoside concentrations in patients undergoing carotid endarterectomy (CEA) under loco-regional anaesthetic. The results showed a significant rise in purins on carotid cross clamping and subsequent return to baseline concentration within 1 h of removal of the clamp and restoration of normal cerebral circulation. This was attributed to increased metabolic stress on the cerebral tissue secondary to diminished blood flow during carotid clamping.

Neurological complications of CEA can be caused by embolism of plaque material from the operative site or cerebral hypoperfusion during carotid cross clamping combined with inadequate collateral perfusion. This can cause significant neurological morbidity. Procedural risk of stroke has been a source of much debate, currently it is quoted at around 2%.

Although some would advocate routine shunting in all patients undergoing CEA to negate this, especially those undertaking general anaesthetic (GA) CEA, the intraoperative use of shunts is not without risk, namely air embolism and vessel wall trauma. Published evidence has failed to clearly advocate the superiority of either selective or routine shunting, ultimately meaning that shunting may be unnecessary in a proportion of cases. Local anaesthetic (LA) affords clinicians the luxury of direct neurological assessment of the awake patient, which is not possible with use of a GA. Many mechanisms of monitoring cerebral perfusion during GA exist including electroencephalography (EEG), somatosensory and motor evoked potentials (SSEP), transcranial Doppler (TcD), stump pressure monitoring and cerebral oximetry. None of these modalities have been universally accepted as they all have drawbacks in application and interpretation of results intraoperatively.

This small, prospective, observational study was designed to replicate the previously published work in a cohort of patients undergoing CEA with GA to investigate the hypothesis that measurement of intraoperative changes in systemic purine concentrations could be a reliable marker for developing intraoperative cerebral ischaemia and as such provide surgeons with valuable information regarding an individual patient’s need for shunt insertion intraoperatively.

Methods

Ethics

Ethical approval was obtained from the West Midlands Research Ethics committee (REC ref: 15/WM/0341. IRAS ID: 189877), clinical trials.gov number: NCT02545166. Patients were identified by the direct healthcare team, approached by the research team and provided with study information at least 48 h prior to the planned procedure. Written consent was then obtained on the day of the procedure.

Patients

LA cohort. Results from the LA CEA group have been published previously. In brief, 17 patients underwent CEA with LA block at a single UK university teaching hospital. All CEA were performed by a single surgeon. Shunt use was at the surgeons’ discretion based on clinical need.

GA cohort. In this study, 40 patients undergoing GA CEA were recruited from two UK university teaching hospitals between March and December 2016. As purine nucleosides had not been studied using this technology previously, a pragmatic approach to recruitment was employed. Recruitment of 40 patients was deemed achievable within a practical time frame based on the expected numbers of patients referred for CEA. All patients were referred from a stroke physician, had undergone imaging to confirm the presence of clinically significant carotid artery disease and were discussed at an appropriate multidisciplinary team meeting (MDT) prior to being offered CEA. Patients with disorders of purine metabolism, such as gout, were excluded and all patients were starved prior to anaesthetic, meaning recent ingestion of protein was not a confounding factor.

Surgical procedures

CEA was performed in the standard format. The use of intraoperative shunting was not dictated by the study design and remained at the surgeons’ discretion. Clinical assessment of the need for shunting varied between recruiting sites. One site routinely employed the use of TcD ultrasound. The indication for shunting was defined as >50% loss of middle cerebral artery velocity. At the second site, the method of determining shunt use was based on the surgeon’s preference. The methods used included incomplete circle of Willis on preoperative computed tomography (CT) or magnetic resonance (MR) angiography, poor back bleeding, low stump pressures or haemodynamic instability on clamping. All patients were managed in a high dependency unit (HDU) setting postoperatively.
Perioperative blood pressure (BP) was regulated between a systolic pressure of 110 and 180 mmHg using glyceryl trinitrate (GTN) or metaraminol as necessary, in order to maintain a mean arterial pressure (MAP) to give adequate cerebral perfusion pressure (CPP). The choice of anaesthetic was the discretion of the anaesthetist.

**Sampling and SMARTchip**

Blood samples for analysis of purine nucleoside concentrations were obtained via an arterial cannula, inserted into the radial artery as part of routine care. Samples were taken prior to skin incision, after carotid artery exposure, at 10-min intervals during carotid clamping and (if applicable) shunt insertion, on restoration of anatomically normal blood flow, 1 h after clamp removal and at 24-h post-clamp removal.

The mechanism of action of the biosensor sensor has been described in detail elsewhere. In brief, a Ruthenium purple sol–gel layer was anchored to a gold-plated electrode. This contains a layer of enzymes sensitive to adenosine, inosine, hypoxanthine and xanthine. Each chip was calibrated using a buffer solution containing 10 µM adenosine before use. Differences in the current produced between the buffer and the blood sample produce an integer that can be interpreted as a purine concentration. Analysis of whole blood samples was carried out immediately after sampling within the theatre suite. Analysis of samples to obtain a purine concentration takes 180 s, allowing near-real-time interpretation of changes in purine concentrations.

**Statistical analysis**

Purine values are presented as medians with 95% confidence intervals. For the purposes of analysis, the GA cohort was divided into two groups: those that required insertion of a shunt (shunted) and those that did not require shunt insertion (unshunted), as cerebral blood flow (CBF) is significantly different with shunt insertion. Although there were set intraoperative sampling time points, these time points do not represent a linear time pattern as individual operative stages were achieved at varying time points. The time points presented are median (with 95% confidence limits) time at which the sample was obtained.

Friedman’s analysis of variance (ANOVA) was undertaken to assess changes within each cohort at the specified sampling points throughout the study period. Mann–Whitney U test was used for comparisons between the previously published LA data and both of the GA group data. Friedman’s ANOVA with Bonferroni correction was used to analyse intragroup MAP changes. A p value of <0.05 was considered to be significant.

**Results**

**Demographics**

A total of 40 patients were recruited to the GA CEA study. On validation of the biosensor data, three patients were subsequently excluded from the analysis due to interference during sampling leading to erroneous readings. The results for the remaining 37 patients are presented here.

The results for 28 males and 9 females with a mean age of 72 (range 41–87) were analysed. Indications for surgery were as follows: stroke (n = 20), transient ischaemic attack (TIA) (n = 15) and isolated ocular symptoms (n = 2). Cardiovascular comorbidity as well as previous cerebrovascular disease was prevalent within the cohort. Full demographics of patient comorbidity are shown in Table 1 with the LA cohort demographics for comparison. In all, 23 patients underwent CEA with no shunt and 14 patients were shunted intraoperatively.

**Operative timings**

Intraoperative timings are shown in Table 2. There was no significant difference in procedural length between the GA shunted and GA unshunted cohorts (147 ± 50 vs 143 ± 32 min, p > 0.05); the LA procedures were significantly shorter than this (95 ± 41 min, p = 0.09 vs shunted and p = 0.04 vs

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**Table 1. Demographics from the recruited GA cohort.**

| Demographic                | Number | p   |
|----------------------------|--------|-----|
| Total participants         | 37     | 17  |
| Sex (M:F)                  | 28:9   | 12:5| ns  |
| Age (mean)                 | 72     | 72  | ns  |
| Indication for CEA         |        |     |
| Stroke                     | 20     | 4   | 0.02|
| TIA                        | 15     | 7   | ns  |
| Amarosis                   | 1      | 3   | 0.06|
| Ocular ischaemic syndrome  | 1      | 0   | ns  |
| Asymptomatic               | 0      | 2   | 0.04|
| Comorbidity                |        |     |
| Hypertension               | 27     | 12  | ns  |
| Previous stroke            | 25     | 6   | 0.01|
| Hyperlipidaemia            | 18     | 10  | Ns  |
| Diabetes                   | 8      | 0   | 0.03|
| Ischaemic heart disease    | 5      | 4   | 0.41|
| Peripheral vascular disease| 2      | 3   | ns  |
| Smoking status             |        |     |
| Current                    | 19     | 4   | 0.04|
| Ex                         | 7      | 3   | ns  |
| Never                      | 11     | 7   | ns  |
| Antiplatelet therapy       |        |     |
| Dual                       | 19     | 6   | ns  |
| Single                     | 18     | 8   | ns  |
| None                       | 0      | 3   | 0.01|
| Statin therapy             |        |     |
| Yes                        | 33     | 9   | 0.001|

GA: general anaesthetic; LA: local anaesthetic; CEA: carotid endarterectomy; TIA: transient ischaemic attack.

Data from the previously published LA cohort is included for comparison.
Table 2. Intraoperative timings by procedure group.

| Procedure group, median (CI) | GA shunted | GA unshunted | LA |
|------------------------------|------------|--------------|----|
| Operative time (min)         | 147 (97–197) | 143 (121–165) | 95 (54–136) |
| Relative ischaemic time (min)| 12 (4–20)   | 49 (49–53)   | 34 (19–49) |

GA: general anaesthetic; CEA: carotid endarterectomy; LA: local anaesthetic; CI: confidence interval.

There was no significant difference in the total operative time in GA CEA; LA CEA was significantly shorter ($p = 0.09$ and $0.04$ vs unshunted and shunted, respectively). As expected, the shunted group experienced a significantly shorter relative ischaemic time ($p = 0.001$ and $0.014$ vs unshunted and LA, respectively).

Intraoperative BP control

Intraoperative MAP profiles for all the groups are shown in Figure 1. In both the LA and GA unshunted groups, intraoperative MAP varied significantly from baseline readings. MAP in the LA cohort was significantly increased after application of carotid clamps ($p = 0.03$), whereas MAP in the unshunted GA cohort was significantly decreased after anaesthesia ($p = 0.006$), though this difference was not sustained throughout the remainder of the procedure. MAP in the GA shunted group was significantly lower than in the LA group ($p = 0.018$). There was no statistically significant difference between the GA cohorts.

Intraoperative changes in peripheral arterial purine concentrations

Purine concentrations at baseline were similar between the GA cohorts: $3.02 \pm 1.11 \, \mu M$ and $3.16 \pm 1.85 \, \mu M$ in the unshunted and shunted cohorts, respectively ($p > 0.05$). These baseline readings were also comparable with baseline in the LA cohort ($2.4 \pm 1.35 \, \mu M$, $p = 0.23$).

There was no significant variation within either of the GA groups from baseline after 30 min of carotid clamping when compared with baseline values: $2.07 \pm 0.89 \, \mu M$ ($p = 0.713$ compared to baseline) and $2.4 \pm 3.09 \, \mu M$ ($p = 0.474$ compared to baseline) in the unshunted and shunted cohorts, respectively. In addition, there was no significant difference in purine concentrations between the shunted and unshunted groups at any of the sampling points. The profiles of purine concentration against time from the shunted and unshunted GA cohorts are presented in Figures 2 and 3.

The previously published LA cohort demonstrated a 2.8-fold increase during carotid clamping: $2.4 \, \mu M$ at baseline rising to $6.7 \, \mu M$ during clamping. This did represent a significant increase compared with both GA cohorts at comparable time points (shunted $2.4 \pm 3.09 \, \mu M$ vs $6.7 \pm 3.4 \, \mu M$ and unshunted $2.07 \pm 0.89 \, \mu M$ vs $6.7 \pm 3.4 \, \mu M$, $p = 0.004$).

No significant difference was noted in the postoperative (both 1 and 24 h) purine concentrations between either of the GA groups and the LA group. In addition, no statistically significant changes in purine concentrations was demonstrated in the differing anaesthetic agents used or with increasing degree of ipsilateral or contralateral carotid stenosis.

Postoperative neurological events

There were no reported periproductive neurological events in either the GA or LA cohorts. Four patients reported transient cutaneous sensation alteration in the distribution of the greater auricular nerve (2 GA and 2 LA).

Discussion

The results reported here from patients undergoing GA CEA were significantly different from the results reported from the previous LA study. The only methodological difference between the cohorts was the anaesthesia chosen under which to perform CEA. From these results, it could be hypothesised that GA may decrease the cerebral cellular metabolic rate so that ischaemic purine nucleoside production is not activated or its production is limited to a point where it can no longer be detected at significant concentrations in peripheral circulation.

Differences in cerebral metabolism between LA and GA CEA have previously been identified; McCleary et al.23 analysed the results of continuous jugular venous oximetry (SjVO₂) and near-infrared spectroscopy (NIRS) in patients undergoing GA or LA CEA. They demonstrated a smaller fall in SjVO₂ in the GA cohort than in the LA cohort (9% vs 13%), meaning cerebral oxygen consumption was decreased to a greater extent in the GA cohort. NIRS measurement (as a proxy marker of cellular ischaemia) showed a greater fall in cytochrome oxidase in the LA cohort, suggesting a greater degree of tissue hypoxia in patients who were conscious during CEA. These findings are consistent with the contrasting findings of our LA and GA studies; that is, cerebral metabolic rate is higher in patients undergoing LA CEA than GA CEA. This in turn may lead to greater cerebral cellular metabolic stress, which is appreciated as a detectable rise in purine nucleosides that is not replicated under GA.

Although the mechanism of action of many anaesthetic agents is not fully understood, it is known that neuronal activity is depressed by GA.24 It is extremely difficult to directly measure cerebral metabolism directly in vivo and studies have used a number of proxy measures, including changes in cerebral uptake of oxygen (CMRO₂) and...
Figure 1. Mean arterial pressure profiles for (a) GA shunted, (b) GA unshunted and (c) LA subgroups. Data are presented as median with 95% confidence limits. Sampling points represent preoperative baseline, post-anaesthetic prior to procedure starting, artery exposure, clamp on and reperfusion. Horizontal error bars during the intraoperative sampling periods represent 95% confidence limits of time point at which each operative stage was reached. Panel (a) represents an additional data point obtained on shunt insertion. No preoperative MAP was obtained for the LA subgroup as there was no GA. MAP was significantly decreased compared with baseline in the GA unshunted cohort ($p = 0.03$). MAP was significantly increased in the LA cohort on application of the carotid clamp compared with baseline ($p = 0.018$). MAP in the GA shunted group was significantly lower than in the LA group ($p = 0.06$).

Figure 2. Chart of purine levels in the GA shunted cohort using median purine concentration (vertical bars represent 95% CI). The time points for each operative stage represent the median time from baseline with 95% CI. Observed changes in purine concentrations were not significant ($p > 0.05$).
relative glucose metabolic rate (rGMR) to identify changes in cerebral metabolic rate caused by anaesthetic agents.\textsuperscript{25,26} Sevoflurane was found to decrease global cerebral GMR by 56\% (p < 0.01).\textsuperscript{27} Propofol induced a decrease in rGMR by 54\% (p < 0.01).\textsuperscript{26} It also induced a total reduction in CBF by 47\%. Xenon was shown to decrease rGMR but increase CBF.\textsuperscript{24} Volatile induction of anaesthesia (VIMA) has also been shown to have less impact on cerebral oxygenation when compared with total intravenous anaesthesia (TIVA).\textsuperscript{28}

In addition to changes in metabolic rate, GA can induce changes in CBF. Under normal physiological conditions, CBF and cerebral glucose metabolism are tightly linked.\textsuperscript{29,30} However, the studies above demonstrate that with many commonly used anaesthetic agents, this coupling is lost.\textsuperscript{25} rGMR has been found to be decreased disproportionately (to a greater extent) when compared with CBF. This leads to the concept of ‘luxury perfusion’,\textsuperscript{26,31,32} which is a phenomenon of oxygen delivery to tissue in excess of metabolic requirement. This can occur due to relative hyperaemia, such as in response to a period of hypoxia or a decrease in cellular metabolic rate, with relatively preserved CBF such as in the case of GA.

The anaesthetic agents mentioned above, or derivatives of them, are all commonly used in current anaesthetic practice and indeed were used to anaesthetise the participants in the study described here. It stands to reason that the decrease in cerebral metabolic rate induced by GA could be equal to or greater than that of the decrease in CBF caused by carotid cross clamping (taking into account supply from collateral vessels) and would lead to a physiological picture mimicking the described ‘luxury perfusion’. Variation in procedure duration and relative ischaemic time also supports the hypothesis that GA has an influence on cerebral purine production. Despite a significantly longer relative ischaemic time than the LA group, the anticipated purine rise was not observed.

Adequate CPP to maintain normal cerebral functioning is defined as being between 50 and 70 mmHg.\textsuperscript{33} This is derived from the difference between MAP and intracranial pressure (ICP). Maintenance of CPP intraoperatively is critical to minimise the risk of ischaemic insult. We have assumed that all recruited patients had normal ICP, and have shown that intraoperative MAP was maintained to prevent malperfusion contributing to ischaemic purine production. The relatively lower MAP readings in the GA shunted cohort may be attributable to less rigorous BP control given cerebral perfusion via the shunt. The relative increase in MAP in the LA study may also be explained by discomfort or anxiety experienced by the patient during an LA procedure.

The use of shunts in carotid surgery remains contentious and there is significant variation in practice globally. Selective, routine and no shunt approaches all have staunch supporters. A Cochrane review published in 2014\textsuperscript{34} involving 1270 procedures and a retrospective study of 28,475 procedures\textsuperscript{35} both concluded that there was insufficient robust evidence to support the use of routine, selective shunting or no shunt policies to reduce stroke risk. The absence of significant purine rises in the GA cohort, compared with the previous LA cohort may indicate that the decrease in cerebral metabolic rate afforded by general anaesthetic agents gives an element of cerebro-protection against clamp-induced cerebral hypoperfusion ischaemia. Therefore, in a proportion of cases, the perceived protection offered by shunting may be unnecessary. A larger study is needed to assess purine nucleoside changes only in patients with evidence of significant cerebral ischaemia intraoperatively and the effects of shunt insertion.

This study has a number of limitations. First, this study is a small proof-of-concept study, with a number of demographic differences between the GA and LA cohorts. This is likely due to the relatively small numbers of patients in each
group, especially the LA cohort. As medical conditions affecting purine production were excluded, the range of comorbidity identified would not have confounded the primary outcome of this study. Second, shunting remained at surgeons’ discretion and therefore varied between surgeons; in addition, determination of the need for shunting varied between surgeons and between the recruiting sites. This potentially serves to confound changes in purine nucleosides as it would be expected that shunt insertion would attenuate ischaemic purine nucleoside production as cerebral flow is maintained. This is demonstrated in the results obtained in patients requiring shunt insertion in the previously published LA study. Given rises in purine nucleosides in the LA cohort were appreciated prior to neurological symptoms, it was not deemed appropriate to require surgeons to deviate from their preferred mechanisms of cerebro-protection. A lack of appreciable purine rise in conjunction with no perioperative neurological events could be interpreted as appropriate prophylactic shunt insertion based on existing selection mechanisms. Third, anaesthetic choice was again not standardised. The aim of this study was not to compare the choice of anaesthetic agent against intraoperative neurological disturbance and the potential significance of anaesthetic choice became apparent during the study period. Future work around purine nucleosides should seek to standardise anaesthetic regimens to avoid this as a potential confounder.

**Conclusion**

While this small study does not demonstrate conclusive evidence that purine nucleosides can be used as a marker of cerebral ischaemia, the comparisons to the LA study offer information about the differences in cerebral ATP metabolism between LA and GA. We hypothesise that the lack of a rise in purine nucleosides in GA CEA may be caused by a decrease in cerebral metabolic rate and loss of metabolic rate-blood flow coupling caused by general anaesthetic agents.

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**Declaration of conflicting interests**

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: N.E.D. is a cofounder of Sarissa biomedical that manufactures SMARTchip. F.T. is an employee of Sarissa.

**Ethical approval**

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**Informed consent**

Written informed consent was obtained from all subjects before the study.

**Trial registration**

Clinicaltrials.gov number: NCT02545166.

**ORCID iDs**

Owain Fisher https://orcid.org/0000-0002-1303-3667
Ruth A Benson https://orcid.org/0000-0001-5889-4391

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