Adenosine triphosphate bioluminescence assay for monitoring contamination of the working environment of anaesthetists and cleanliness of the operating room

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Abstract

Anaesthetists possibly contribute to the spread of infections during anaesthesia. The adenosine triphosphate (ATP) bioluminescence assay is an easy-to-perform, on-the-spot assay that provides objective data; therefore, using the LuciPac®Pen and the Lumitester PD-20®System, we assessed contamination of the working environment of anaesthetists before and after surgery as well as their hands at the time of each procedure during induction and extubation. Similarly, cleanliness of the operating room was evaluated using this assay to determine whether it is useful to assess the effectiveness of the routine cleaning protocols followed after surgery. ATP concentrations in the working environment of anaesthetists and their hands increased during surgery. In addition, ATP concentrations within the working environment decreased after routine cleaning with ethanol or accelerated hydrogen peroxide; however, there were no differences in the number of sites with ATP concentrations >500 relative light units before and after cleaning. This method is useful to evaluate contamination of the working environment of anaesthetists; nevertheless, it is prudent to evaluate the effectiveness of routine cleaning protocols because ATP bioluminescence assays are influenced by the use of various disinfectants at varying concentrations.

Keywords

Adenosine triphosphate bioluminescence assay, hands of anaesthetists, routine cleaning, working environment of anaesthetists

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Introduction

Healthcare associated infections (HAIs) have been linked to contaminated hospital environments, such as the operating room (OR), and they contribute to increased patient morbidity, patient mortality and healthcare costs (Wenzel, 2007; Anderson et al., 2008). Of late, there has been a high risk of infection in operations on elderly people who have many complications and operations that take a long time (Turrentine et al., 2006; Procter et al., 2010). During general anaesthesia, potentially pathogenic organisms can be transmitted between patients and instruments used for anaesthesia through the hands of anaesthetists because anaesthetists perform procedures that may expose them to blood and/or body fluids (Loftus et al., 2008, 2011). We

previously reported the contamination of keyboards in the OR and that the wet, sticky gloves of anaesthetists can easily transmit bacteria (Fukada et al., 2008). Thus, anaesthetists are probably involved in the spread of HAIs; however, many anaesthetists may be unaware that they contribute to contamination in the OR during general anaesthetists. Furthermore, the cleaning staff are required

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to clean the operating table and instruments quickly using predetermined disinfectants to accommodate serial surgeries. Therefore, it would be important for anaesthetists to recognise that they may transmit pathogenic organisms surrounding their working environments, and for cleaning staff to provide feedback regarding the effectiveness of their cleaning protocols.

The degree of environmental contamination and cleanliness in hospitals was previously evaluated using visual assessment and microbiological culture swabs. However, the adenosine triphosphate (ATP) bioluminescence assay has been used recently (Cooper et al., 2007; Lewis et al., 2008; Andersen et al., 2009; Boyce et al., 2009, 2010). ATP measurement was originally used to evaluate the degree of contamination or cleanliness in the food and beverage industry. The LuciPac®Pen and the Lumitester PD-20®System (Kikkoman Biochemifa Co., Tokyo, Japan) are specifically designed to evaluate contamination on the surfaces of instruments by measuring concentrations of ATP and adenosine monophosphate (AMP), a product of natural ATP hydrolysis, which is considered more prevalent in certain types of dirt. These devices detect concentrations that are expressed as the number of relative light units (RLU). However, there are no studies on contamination of the surroundings of anaesthetists and of instruments used for general anaesthesia based on the ATP bioluminescence assay; therefore, the primary aim of this study was to evaluate contamination at certain sites in the working environment of anaesthetists that are touched many times by these individuals, particularly during serial surgeries. The secondary aim was to evaluate whether this method would be useful to determine the effectiveness of routine cleaning procedures between surgeries in the OR.

Methods

Surfaces with potential ATP contamination were evaluated three times using the LuciPac®Pen and the Lumitester PD-20®System. The LuciPac®Pen is a kit that combines reagents with the swab required for picking up ATP from the test medium: the investigator (TF) took the swab from the kit, moistened it with sterilised water, uniformly wiped every direction of the objects without the dried part, then returned the swab to the LuciPac®Pen, shook it well for reaction with the luminescent reagent and inserted the LuciPac®Pen into the measurement chamber of the Lumitester PD-20® apparatus. The benchmark of ATP concentration used to evaluate hospital cleanliness varies depending on the devices. According to the manufacturer, the benchmark for contamination detection on non-critical instruments using these devices ranges from 200 RLU to 500 RLU and that for contamination detection on the dominant hand is ≤ 1000 RLU; therefore, we considered the working environment of anaesthetists and their dominant hand to be clean if benchmarks were ≤ 500 and ≤ 1000 RLU, respectively.

ATP concentrations on the instruments used for anaesthesia and the dominant hands of anaesthetists were measured during surgeries that required tracheal intubation and maintained anaesthesia by venous anaesthetics or volatile anaesthetics for approximately 4 h, such as orthopaedic, endocrine, gynaecological, plastic, and bowel surgeries. In our OR, anaesthetists and nurses have to wear non-sterile gloves from the time the patient lies on the operating table to the beginning of the operation and also from the end of an operation until the time the patient leaves the OR as long as their gloves are not contaminated. If their gloves get contaminated, they have to change and wear new non-sterile gloves. During an operation, anaesthetists wear non-sterile gloves when they touch patients. They are prohibited from reusing gloves. In this study, anaesthetists wore sterile gloves instead of non-sterile gloves during the induction and extubation periods (approximately 30 min each). The reason for measuring ATP concentration on the gloves of the anaesthetists' dominant hands was that anaesthetists usually operate their instruments, including the computer mouse, using their gloved dominant hand. Regarding the working environment of anaesthetists, ATP concentrations on the control knob and adjustable pressure-limiting (APL) valve of the anaesthesia machine (Aisys GE Healthcare Japan Co. Ltd., Tokyo, Japan), the control panel of the syringe pump (Terumo Corp., Tokyo, Japan), and the numeric keys of the keyboard/two-button mouse used for performing anaesthetic recordings were measured before and after surgery and after cleaning OR (Figure 1). The keyboard examined was the general keyboard, which was not waterproof, was not a flat type and had no cover. The measurement time points were defined as follows: 'before surgery' was approximately 30 min before the patient entered the OR, and the abovementioned sites were thoroughly wiped with microfibre sheets that contained 76.9%–81.4% ethanol (Shodokku® Super, Hakujiji Co. Ltd., Tokyo, Japan) and then dried. These sites were considered 'clean'. 'After surgery' was immediately after the surgery and after the patient had left the OR, but before the sites were cleaned. 'After cleaning' was approximately 30 min after cleaning performed by three to four members of the cleaning personnel who spent approximately 10–15 min wiping the sites using several sheets of Shodokku® Super or 0.5% accelerated hydrogen peroxide (Hyprox Accele Wipes™, Virox Technologies Inc. Oakville, Ontario, Canada), and then allowed the sites to dry. If surfaces appeared visibly soiled by materials such as blood, they were cleaned with a new sheet after the removal of the material by a sheet at one swipe. The number of sheets used and the method used to wipe the sites were left to the cleaning personnel's preferences. 'Induction' was defined as the time between the entrance of the patient into the OR to the point of intubation using a tracheal tube, whereas 'extubation' was defined as the time from the end of surgery to the extubation of the tracheal tube and the departure of the patient from the OR.

Figure 1. ATP measurement sites within the working environment of anaesthetists (a) control knob and (b) adjustable pressure limiting (APL) valve of the anaesthesia machine; (c) numeric keys of keyboard and mouse; (d) control panel of syringe pump.

Table 1. Changes in ATP concentrations within the working environment of anaesthetists.

(24 surgeries)	Before surgery	After surgery	<i>p</i> value
Keyboard; RLU	179 (154)	632 (432)	< 0.0001
Mouse; RLU	189 (142)	1866 (930)	< 0.0001
APL valve; RLU	261 (142)	1832 (1748)	0.0585
Control knob; RLU	272 (235)	2226 (1661)	< 0.0001
Syringe pump; RLU	140 (90)	2696 (6632)	0.0783

Values are expressed as the mean (SD).

RLU, relative light units.

APL valve, adjustable pressure-limiting valve.

To evaluate contamination of the environment of the OR, we measured pre-operative and postoperative ATP concentrations on the aforementioned sites as well as those on the dominant hands of anaesthetists immediately after wearing sterile gloves and at the time of each procedure during induction and extubation. To evaluate the effectiveness of the cleaning methods using either ethanol or accelerated hydrogen peroxide, ATP concentrations within the working environment of anaesthetists were measured after surgery and after cleaning.

After cleaning, to examine whether ATP concentration was affected by the use of Shodokku® Super or Hypox Accele Wipes™, the working environment of anaesthetists was swabbed with cotton swabs moistened with sterile saline, which were then washed in 1 mL of brain heart infusion and incubated at 37°C for 48 h. To evaluate the influence of 76.9%–81.4% ethanol or 0.5% accelerated hydrogen peroxide on the ATP bioluminescence assay, ATP concentrations of control panels of the syringe pumps, in which

the ATP concentration was 44 (15) RLU when wiped with tap water, were measured after wiping using sheets of Shodokku® Super or Hypox Accele Wipes™.

Data are expressed as the mean (standard deviation; SD), and differences in ATP concentrations at sites between the time of measurement or the effect of cleaning by various disinfectants were analysed using *t*-tests or the chi-square test; those of the gloves of anaesthetists and those of the effect of cleaning were analysed using Dunnett's test. A *p* value of <0.05 was considered statistically significant.

Results

Contamination and the effect of cleaning were evaluated for 24 surgeries in which the patients were anaesthetised by residents or senior residents. ATP concentrations were significantly increased (>500 RLU) at sites in the working environment of anaesthetists after surgery (Table 1). ATP concentrations on the dominant hands of

Table 2. Changes in ATP concentrations by cleaning with ethanol or accelerated hydrogen peroxide.

	Cleaning with ethanol (12 surgeries)			Cleaning with accelerated hydrogen peroxide (12 surgeries)		
	After surgery	After cleaning	p value	After surgery	After cleaning	p value
Keyboard; RLU*	691 (566)	454 (407)	0.0519	573 (249)	533 (512)	0.7133
Mouse; RLU	2206 (1124)	921 (574)	0.0068	1963 (691)	1311 (850)	0.0172
APL valve†; RLU	5167 (10745)	980 (1106)	0.1675	1637 (1242)	1127 (710)	0.0634
Control knob; RLU	1361 (778)	601 (357)	0.0008	2970 (1985)	1119 (919)	0.0005
Syringe pump; RLU	3742 (9356)	547 (688)	0.2405	1609 (1494)	1210 (1303)	0.3195

Values are expressed as the mean (SD).

RLU, relative light units.

APL valve, adjustable pressure-limiting valve.

Table 3. Changes in the number of sites with ATP concentrations >500 RLU after cleaning with ethanol or accelerated hydrogen peroxide.

	Cleaning with ethanol (12 surgeries)			Cleaning with accelerated hydrogen peroxide (12 surgeries)		
	After surgery	After cleaning	p value	After surgery	After cleaning	p value
Keyboard	5	3	0.665	5	4	1
Mouse	12	11	1	12	9	0.217
APL valve	10	6	0.1939	9	8	1
Control knob	12	8	0.1003	12	10	0.4602
Syringe pump	9	4	0.1013	11	7	0.1573

APL valve, adjustable pressure-limiting valve.

anaesthetists increased from 52 (28) RLU immediately after wearing sterile gloves to 86,282 (154,038) RLU ($p = 0.0157$) during induction and to 21,286 (26,821) RLU ($p = 0.7081$) during extubation. APL valves of the anaesthesia machine, control panel of the syringe pump, computer mouse and the hands of some anaesthetists were visibly contaminated by blood and body fluids.

Cleaning with ethanol or accelerated hydrogen peroxide significantly decreased ATP concentrations at some measurement sites; however, there were no differences in the number of sites with ATP concentrations >500 RLU before and after cleaning (Tables 2 and 3).

After cleaning using Shodokku® Super or Hyprox Acele Wipes™, 60% or 68% of the measurement sites, respectively, became negative for bacteria although ATP concentrations increased to >500 RLU; and 100% or 86% of the measurement sites, respectively, became negative for bacteria although ATP concentrations increased to <500 RLU, respectively. After wiping with sheets of Shodokku® Super or Hyprox Acele Wipes™, ATP concentrations of control panels of the syringe pumps increased to 162 (106)

RLU ($p = 0.0738$ vs tap water) or 361 (313) RLU ($p = 0.0120$ vs tap water), respectively.

Discussion

Visual assessment, microbiological culture using swabs and ATP bioluminescence assay are commonly employed to evaluate the environmental contamination and cleanliness in hospitals (Cooper et al., 2007; Lewis et al., 2008; Andersen et al., 2009; Boyce et al., 2009, 2010). Although visual assessment is a simple and subjective method, the values obtained cannot be quantitatively expressed, and the method is insufficient to evaluate invisible organic contamination (Cooper et al., 2007). Microbiological screening is expensive and bacterial culture is time consuming; therefore, these two methods are impractical for immediate feedback. In contrast, the ATP bioluminescence assay can be used to measure viable bacteria and dirt, which is a cause of microbial growth, within 1 min. ATP can be hydrolysed to adenosine diphosphate or AMP. The ATP bioluminescence assay measures ATP concentrations based on the

'firefly principle' in which ATP reacts with luciferin in the presence of luciferase to yield AMP and produce light. Among ATP-measuring devices, only the LuciPac®Pen and the Lumitester PD-20®System can convert AMP into ATP and measure AMP concentration as ATP concentration; therefore, this system reflects the presence of viable bacteria and dirt more accurately. However, it is important to note that, at the time of ATP measurement, a correlation between ATP concentration and bacterial count is not always observed; additionally, the development of an ATP benchmark to evaluate hospital cleanliness is dependent on the measuring devices; thus the comparison of ATP values measured using different devices is not possible (Brown et al., 2010; Carmen et al., 2012).

We found that some anaesthetists touched their working environment with gloves that were contaminated with blood or sticky substances such as the secretion of patients or drugs containing glucose. We previously reported that the keyboards of systems that store anaesthetic records were contaminated, as confirmed by microbiological swab cultures (Fukada et al., 2008). However, based on microbiological culture, we were not able to offer immediate feedback to anaesthetists by showing that their behaviour was a potential source of contamination, because it takes approximately two days to obtain these results. Conversely, the ATP bioluminescence assay is completed within 1 min and can evaluate contamination of the working environment of anaesthetists. After surgery, ATP concentrations increased to >500 RLU at the measurement sites, although contamination was not uniform. The level of ATP concentration depended on the design of the instruments, operating methods, frequency of use, and setting positions. For example, the surface of the APL valve was uneven, whereas that of the control knob was rough, and these sites were firmly operated using two or more fingers. The mouse comprised several parts and had exposed grooves, which were easily contaminated; furthermore, it was necessary to grasp and operate the mouse using the entire hand. The control panel of the syringe pump was smooth; however, it was easily contaminated by blood or drugs because it was located under the intravenous solution and near the three-way stopcocks. Because the numeric keys of the keyboard were pressed using the fingertips, there may have been little contamination at this site compared with other sites. Our results showed that ATP concentrations within the working environment of anaesthetists increased after surgery. This indicates that the postoperative ATP concentration within the working environment of anaesthetists probably increases by transmission through the hands of anaesthetists; therefore, contamination of the hands of anaesthetists is a contributing factor to contamination of the OR, further indicating the importance of hand hygiene to prevent the spread of HAIs.

Cleanliness in the OR is considerably influenced by the skill of the cleaning staff, the types of disinfectants used, cleaning duration, design and quality of the cleaning

equipment and degree of contamination of the visually inspected instruments. In our OR, three to four members of the cleaning personnel spent approximately 10–15 min cleaning the equipment immediately after surgery, in preparation for the next surgery, using several sheets containing ethanol or accelerated hydrogen peroxide, as per the recommendations of the infection control team. ATP concentrations on most of the instruments for anaesthesia tested decreased after cleaning, despite ATP concentrations being >500 RLU, and no differences were noted in the number of sites with ATP concentrations >500 RLU after surgery and after cleaning. However, more than 60% of the measurement sites in which ATP concentrations were >500 RLU became negative for bacteria by BHI turbidity after cleaning. The reason for the absence of differences in ATP concentrations between before and after cleaning, despite being negative for bacteria, was the effect of the ingredients in the cleaning products contained in the sheets used to perform the ATP bioluminescence assay. ATP concentrations are influenced by the use of various disinfectants and their varying concentrations; however, there are no data regarding the use of 0.5% accelerated hydrogen peroxide (Green et al, 1998, 1999; Calvert et al, 2000). ATP concentration decreased when using Shodokku® Super; however, these results may be affected by different ethanol concentrations and sheets. The effect of 0.5% accelerated hydrogen peroxide on ATP concentrations is unclear. Therefore, we must be cautious when evaluating contamination using the ATP bioluminescence assay after cleaning, to ensure complete removal of pathogenic organisms.

There were some limitations to our study. We did not investigate the correlation between postoperative contamination and infection because we evaluated ATP concentrations during a limited number of surgeries. Moreover, we did not investigate the influence of various disinfectants on the ATP bioluminescence assay results.

In conclusion, the ATP bioluminescence assay is a useful method to evaluate the spread of contamination through the hands as well as the working environment of anaesthetists, which renders it useful to provide feedback to anaesthetists regarding their contamination footprint; however, it is also prudent to evaluate the effectiveness of routine cleaning protocols.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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