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Virucidal activity of formaldehyde solutions used for preservation of allograft tympano-ossicular systems

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ABSTRACT

Objective: Allograft tympano-ossicular systems (ATOS) can provide superior outcomes in particular circumstances, for example, in case of a need for total reconstruction of the eardrum and chain. ATOS are preserved in a 2.7%–4% formaldehyde solution after procurement at room temperature (15-25°C) for 2–5 days, followed by 4 °C for a total storage of at least 14 days. This study aimed to review the literature on the virucidal effect of formaldehyde on viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: Narrative review of the literature available on the virucidal effect of formaldehyde, as searched in the scientific database PubMed.

Results: Both free and intracellular HIV as well as HBV and HCV are significantly reduced at low concentrations of formaldehyde and short exposure time. Factors increasing the effectivity of formaldehyde solutions are high concentration, long exposure time, and high temperature. It has been demonstrated that HIV-infected allografts are disinfected by formaldehyde preservation. No case of HIV, HBV, or HCV transmission through ATOS has been reported. Coronaviruses closely related to SARS-CoV-2, such as SARS-CoV and MERS-CoV, are inactivated by low concentrations of formaldehyde solution, even at short exposure times.

Conclusion: These findings indicate that formaldehyde is effective in inactivating HIV, HBV, HCV, and coronaviruses. ATOS are stored in a high concentration formaldehyde solution for a long period. The applied preservation method of ATOS, including temporary storage at room temperature, should be maintained for effective inactivation. The formaldehyde preservation method in combination with donor screening and serological and nucleotide amplification testing make ATOS a very safe reconstruction material.

Keywords: Ear ossicles, formaldehyde, tympanic membrane, tissue banks, virus inactivation

Introduction

The first clinical application of allogeneic ossicles for middle ear reconstruction purposes in chronic otitis media was established by Jean Marquet in 1963 (1). The main advantage of allograft tympano-ossicular systems (ATOS) is their ability to restore the anatomy of completely disrupted middle ears, enabling the surgeon to fully resect tissues afflicted by chronic otitis media. Moreover, ATOS demonstrated excellent biocompatibility, even in chronically infected environment. However, concerns on safety, especially regarding transmissible diseases, for example, human immunodeficiency virus infection (HIV), limited its use. When the relationship between variant Creutzfeldt–Jakob disease (CJD) and bovine spongiform encephalopathy (BSE) became obvious, measures taken during the BSE endemy led to the discontinuation of ATOS in many countries because of the dura mater contact during the procurement (2). Meanwhile, the new European Union (EU) regulations on tissues and cells assure maximum quality, traceability, and safety procedures to reduce the risk of transmissible diseases. Currently, disease transmission is a hot topic because of the coronavirus disease 2019 (COVID-19) pandemic, and to date, little is known regarding potential severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission through tissue implantation.

Recently, Van Rompaey et al. (3). demonstrated that running a tympano-ossicular tissue bank, complying with national

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and EU regulations, is possible and critical to ensure that the patient will receive allograft tissue that is safe, individually checked, and prepared in a suitable environment. In contrast to the old transcranial procurement technique, the switch to an endoscopic procurement technique, which avoids opening the skull and contact with intracranial tissue and liquid, has eliminated the risk of prion disease transmission by the use of ATOS (4).

One of the selection criteria for potential donors is the absence of a medical history or risk factors related to HIV, hepatitis B virus (HBV), or hepatitis C virus (HCV). In addition, each potential donor's blood sample is screened for anti-HIV-1 antibodies, anti-HIV-2 antibodies, HBs antigen, anti-HBc antibodies, anti-HCV antibodies, and syphilis. In many countries, such as Belgium, nucleic acid amplification testing (NAT) is performed for HIV-1 and 2, HBV, and HCV. However, NAT in Belgium is not enforced by law in case of an inactivation step validated for the concerned viruses.

This study aimed to review the available literature on inactivation of HIV, HBV, HCV, and SARS-CoV-2 by formaldehyde solutions.

Methods

Literature search was performed using the scientific search engine PUBMED. Reports on the virucidal effect of formaldehyde solutions on HIV, HBV, HCV, and coronaviruses were included. Specifically, reports with primary data were considered, and systematic reviews and opinions were excluded.

Results

Formaldehyde

Formaldehyde is a soluble gas in water with the chemical structure CH_2O. At higher concentrations, the diluted solutions are biocidal and destroy all viable cellular organisms. Even at a 1% concentration, formaldehyde is bactericidal, fungicidal, tuberculocidal, and virucidal. However, the presence of organic material reduces its effect. Temperature also significantly affects the sterilizing effect of formaldehyde solution: the rate of inactivation increases on raising the temperature until 70 °C–80 °C is reached (5).

Main Points:

- Allograft tympano-ossicular systems (ATOS) are preserved in a 2.7%–4% formaldehyde solution at room temperature for 2–5 days, followed by 4 °C for a total storage period of at least 14 days.
- Multiple studies have shown that HIV, HBV, and HCV are inactivated by immersion in a formaldehyde solution of lower concentrations at much shorter exposure time than the one used.
- Viruses closely related to SARS-CoV-2, such as SARS-CoV and MERS-CoV, are inactivated by lower concentrations at lower exposure time, indicating that a similar effect on SARS-CoV-2 may be expected.
- Immersion in a formaldehyde solution adds an extra layer of safety on the existing safety measures by closing the detection window phase of serological and nucleotide amplification testing (NAT) and its effect on viruses in general. However, it does not replace NAT, because the virucidal effect of formaldehyde solution on possibly very high viral loads has not been proven.

Formaldehyde annihilates microorganisms and inactivates autolytic enzymes. Furthermore, it alters the molecular structure of proteins: intra- and intermolecular bridges are created between collagen molecules, thereby impeding the effect of proteolytic enzymes (6). This physiological mechanism probably explains the antimicrobial activity of formaldehyde (7).

Formaldehyde has a significant influence on denaturation of DNA induced by warmth or acid. If DNA is exposed to formaldehyde before denaturation, it is more resistant to denaturation by forming chromatin bridges. If formaldehyde exposure starts during denaturation, the process is accelerated by its passive denaturing effect (8).

HIV

Several groups have studied the effect of different formaldehyde concentrations on HIV. The studies of different research groups are listed in Table 1.

Majoor et al. (9). mentioned several studies that confirmed that a 4% formaldehyde solution provided HIV disinfection, and HIV transmission has never been observed in patients receiving ATOS (although it has been observed when using allograft dura).

Lubbe and Fagan (10) hypothesized that owing to the compact nature of the ossicular bone and the fact that HIV is preferably found in lymphoid tissue and monocytes, viral load in ossicles is extremely low.

According to the World Health Organization (WHO), a 4% formaldehyde solution is an effective HIV disinfectant (11). Their infection control manual does not, however, specify the necessary exposure time and temperature.

HCV

Hepatitis C is a widespread disease affecting 170 million people worldwide and causes severe illnesses, including cirrhosis and hepatocellular carcinoma. The results of 2 experiments are presented in Table 2.

HBV

Hepatitis B affects 350 million people globally. The results of 2 experiments are presented in Table 3.

Coronaviruses

SARS-CoV-2 is a new coronavirus that was transmitted from animals to humans in China at the end of 2019, resulting in the COVID-19 pandemic (12). As SARS-CoV-2 is currently a very new virus, there are no studies yet on the effect of formaldehyde on it. However, the virus is similar to the SARS-CoV virus, which caused the SARS outbreak in 2008, and the Middle East respiratory syndrome coronavirus (MERS-CoV), which emerged in April 2012. Studies have shown that SARS-CoV-2 belongs to the same genus as SARS-CoV (betacoronavirus) and shows around 80% genetic similarity to SARS-CoV and around 50% similarity to MERS-CoV (13). The effect of formaldehyde on the latter viruses has been studied and is listed in Table 4.

Table 1. Overview of studies on the effect of formaldehyde on HIV

Group	Sample	Formaldehyde solution	Incubation time	Incubation temperature	Results
Spire et al.[22]	Free HIV originating from infected T-lymphocyte cell cultures	0.037%–0.04%	2 hours	Not specified	This relatively low concentration reduces reverse transcriptase activity, which metabolizes RNA into DNA and is commonly used as a biomarker for viral activity. However, reverse transcriptase activity was only reduced to 39% of its baseline activity after 2 hours of immersion demonstrating this concentration's inadequacy to inactivate HIV.
Martin et al.[23]	Free HIV with ID^{50} of 10^5	0.37%–0.4%	5 minutes	Room temperature	A reduction of at least 10^5 of the ID^{50} value.
Cory et al.[14]	H9 cells (embryonic stem cells derived from human blastocysts)	1.85%	30 minutes	4 °C	HIV infectivity reduction was estimated at a factor $10^{4,8}$ (over 99.99% reduction). Reverse transcriptase activity was used as an outcome measure.
Rossio et al.[24]	Free HIV-1 (ID^{50} $4.3x10^3$ to $2.1x10^4$)	0.4625%	25 hours	37 °C	No active virus could be detected afterwards.
Aloisio and Nicholson[15]	PHA-blast cell cultures infected with HIV	1% paraformaldehyde (a polymer of formaldehyde)	1, 3, 6, or 18 hours	4 °C or 37 °C	Inactivation was demonstrated to be slower at 4 °C than at 37 °C. An important but incomplete virus reduction was noted after 18 hours. A control study was set up with 1% formalin (0.37% formaldehyde), but similar results were reported.
Meylan et al.[25]	ATOS obtained from 2 patients infected with HIV	5% formaldehyde	24 hours	Room temperature	No viral load was found in the ATOS. The procurement method (transcranial or transmeatal) was not specified. When studying other infected tissues, a reduction in viral load exceeding 10^5 was observed.
Janssens de Varebeke et al.[2]	ATOS samples procured from symptomatic AIDS patients, presenting with a high viral load	4%	2 weeks	4 °C	Although the control ATOS samples (which were not fixed in the formaldehyde solution) presented with HIV-1 DNA in 3 of 5 cases, no HIV-1 DNA could be detected in preserved samples. The authors concluded that HIV-1 transmission is avoided by immersion in formaldehyde. However, the human globin gene is not destroyed, which leads to the theoretical possibility of intact cellular DNA. An important limitation of this study is its small sample size, which does not allow any relevant statistical analysis.[26]
Buija et al.[27]	Soft tissues, such as the brain and spleen, cartilage, perichondrium, trachea, and blood, procured from 6 patients infected by HIV	4% (samples were stored in Merthiolate or Cialit afterwards)	7 days	Not specified	Samples were analyzed using PCR at baseline storage and after 42 days of storage. Traces of HIV DNA were found in baseline samples of the spleen, perichondrium, trachea, brain, and blood, but not in cartilage. HIV DNA could still be detected in the 4 groups after treatment. Whether the virus was in an inactivated state was not studied.

ATOS: Allograft tympano-ossicular systems, HIV: Human immunodeficiency virus

Table 2. Overview of studies on the effect of formaldehyde on HCV

Group	Sample	Formaldehyde solution	Incubation time	Incubation temperature	Results
Song et al.[28]	HCV culture	0.037%	3/4 hours	Room temperature	No residual activity could be measured after 3 hours of immersion. The addition of normal human serum to the formaldehyde solution resulted in the absence of residual infectivity after 4 hours, demonstrating that a formaldehyde solution of low concentration can inactivate HCV even in the presence of human serum.
Tabor and Gerety[17]	0.1 mL samples of serum containing HCV of documented infectivity	0.037%	96 hours	37 °C	The samples were administered to 3 chimpanzees after incubation in formaldehyde. No recognizable non-A, non-B hepatitis developed in these chimpanzees for 7 months (normal liver histology in liver biopsy, absence of non-A, non-B hepatitis-associated antigen and antibodies, normal aminotransferase levels in weekly serum samples). After this period the chimpanzees were exposed to untreated non-A, non-B hepatitis infected serum. Non-A, non-B hepatitis infection symptoms occurred afterwards (increased aminotransferase and histologic signs of hepatitis in liver biopsy samples).

HCV: Hepatitis C virus

Table 3. Overview of studies on the effect of formaldehyde on HBV

Group	Sample	Formaldehyde solution	Incubation time	Incubation temperature	Results
Sauerbrei et al.[29]	Duck HBV	0.7%	30 seconds, 2, 5, and 15 minutes	Not specified	After 30 seconds, the viral load in the congenitally infected ducks had decreased 3-fold, and 2 minutes after the addition of formaldehyde, the viral concentration was one-tenth of the original concentration. The concentration had reduced 100 times after 5 minutes and 1000 times after 15 minutes. When using D2 cells (a transfected cell line), the viral load had decreased by 100 after 30 seconds. After this time point, there was no further decrease in viral concentration.
Tabor et al.[16]	Samples of human serum with a 10^3 to 10^5 ID50 of HBV	0.00925%	72 hours	37 °C	The samples were administered to chimpanzees after incubation in formaldehyde. No hepatitis B infection was detected during the first 6 months after administration, although it was observed that an ID^{50} of $10^{4,5}$ was not completely inactivated in the presence of 2 mg/mL serum proteins, probably because the serum proteins interfered with the inactivating properties of formaldehyde. Another group of chimpanzees received untreated HBV; in this group, HBs antigen and HBs antibody were detected.

HBV: Hepatitis B virus

Other viruses

Research on the effect of formaldehyde solutions on other viruses is shown in Table 5.

Discussion

The results of Cory et al. (14) indicate that it is more difficult to inactivate intracellular HIV than free HIV. The studies also demonstrate that viral inactivation occurs faster at higher temperatures (15). The effect of formaldehyde on the inactivation of the studied viruses increases with higher temperature, concentration, and exposure time. ATOS are stored in a 2.7%–4% formaldehyde solution at room temperature for 2–5 days, followed by storage at 4 °C for a total exposure time of at least 2 weeks. At this concentration, free HIV will be inactivated quickly. The minimal storage time of 14 days is 700 times greater than that in the study of Cory et al. (14); therefore, a concentration

Table 4. Overview of studies on the effect of formaldehyde on coronaviruses

Group	Sample	Formaldehyde solution	Incubation time	Incubation temperature	Results
Darnell et al.[30]	Supernatant of a SARS-CoV infected cell line, containing a concentration of $10^{6.33}$ virus	0.009%	3 days	4 °C, 25 °C, and 37 °C	The formaldehyde was unable to completely inactivate the virus at 4 °C after 3 days (10^3 reduction), but at 25 °C and 37 °C the virus was almost completely inactivated (10^5 reduction).
Rabenau et al.[31]	SARS-CoV infected cells	0.7% and 1%	2 minutes	Room temperature	This reduced the infectivity by a minimum reduction factor of 103. The authors concluded that this treatment rendered the virus non-infectious.
Kariwa et al.[32]	Supernatant of a SARS-CoV infected cell line	3.5% paraformaldehyde	5 minutes	Room temperature	Reduction of $10^{3.7}$ of infectious virus.
Kumar et al.[33]	MERS-CoV infected cells	4% paraformaldehyde	10/30 minutes	Room temperature	After 10 minutes of treatment, there were still positive cells remaining. After 30 minutes of treatment, a complete inactivation of MERS-CoV could be achieved.
Saknimit et al.21	Coronavirus infected cells	0.7%	10 minutes	Room temperature	Different animal coronaviruses (mouse hepatitis virus and canine coronavirus) are inactivated (reduction factor $10^{3.45}$).

SARS-CoV: Severe acute respiratory syndrome coronavirus, MERS-CoV: Middle East respiratory syndrome coronavirus

Table 5. Overview of studies on the effect of formaldehyde on other viruses

Group	Sample	Formaldehyde solution	Incubation time	Incubation temperature	Results
Al Khleif et al.[34]		0.25%–1%	30–120 minutes	20 °C	Formaldehyde was administered until a reduction in viral load of 10^4 occurred. This showed that formaldehyde has a virucidal effect and that non-enveloped viruses require a higher concentration of formaldehyde and longer exposure time than enveloped viruses.
Moller et al.[35]	Cells infected with 1 complex, enveloped virus (vaccinia virus) and 2 non-enveloped viruses (murine norovirus and human adenovirus)	2%	0.5–168 hours	4 °C, 25 °C, 37 °C	The human adenovirus was fully inactivated after 1 hour exposure, both at 4°C and 25°C. Murine norovirus was fully inactivated after 1 hour exposure at 25°C but not after the same exposure time at 4°C. Vaccinia virus could not be completely inactivated at 4°C, even after 1 week exposure. At 25°C, vaccinia virus was rendered non-infectious within 24 hours.

of 10 to the power of 4.8 intracellular HIV will be inactivated. Moreover, the formaldehyde concentration (2.7%–4%) used is higher. HIV can primarily be found in lymphoid tissue and monocytes; hence, a high viral load is not expected in ATOS. In the study of Janssens de Varebeke, (2) no HIV DNA was detected in treated ATOS when compared with untreated allografts. To date, no cases of HIV transmission through ATOS have been reported. These data indicate that the current formaldehyde treatment inactivates any possible HIV in the allografts. It is expected, but not proven, that very high virus concentrations are also inactivated by formaldehyde treatment.

The concentration needed to inactivate HBV and HCV in the studies of Tabor and Gerety (16, 17) is much lower than that needed to inactivate HIV; therefore, these viruses are more sensitive to formaldehyde treatment. Since ATOS are preserved in a higher concentration of formaldehyde, we can conclude that any possible HBV or HCV virus in the allografts will be inactivated.

The currently widespread SARS-CoV-2 has put an enormous pressure on healthcare systems and forced hospitals to continuously reinvent themselves and postpone treatments, such as elective surgery (18, 19). Tissue banks were also impacted as tissue procurement had to be temporarily discontinued or significantly decreased because of additional donor selection criteria specific for SARS-CoV-2 (20). No studies could be found yet on the effect of formaldehyde on SARS-CoV-2. Rothan et al. (13) showed the genetic similarity of SARS-CoV-2 with other coronaviruses, SARS-CoV and MERS-CoV. The effect of

formaldehyde on these known viruses has been studied. Lower concentrations of formaldehyde than the solution used for ATOS preservation rendered both SARS-CoV and MERS-CoV non-infectious at room temperature when applying shorter exposure times. An older study showed that some animal coronaviruses (mouse hepatitis virus and canine coronavirus) were inactivated by treatment with 0.7% formaldehyde for 10 min at room temperature (21). These findings suggest that if ATOS would be infected by a betacoronavirus such as SARS-CoV-2, the virus will be inactivated by formaldehyde preservation because of the relatively high concentration and long exposure time. The temporary preservation at room temperature has a positive effect on the inactivation of the virus.

The studies on other viruses indicate that even a complex virus such as vaccinia loses its infectivity by exposure to formaldehyde at the concentration, exposure time, and temperature used for ATOS.

Formaldehyde solutions were reported to significantly reduce all studied viruses. Reductions of between log 3 and log 4,9 have been shown in short exposure times (between 30 min and 3 days) at 4 °C and even faster at room temperature. The concentration used to preserve allografts is higher than the concentrations of the reported formaldehyde solutions, and the exposure time is much longer than that in the reported studies. In addition, transmission of HIV, HBV, or HCV through ATOS transplantation has never been reported. Janssens de Varebeke demonstrated that transmission of HIV through ATOS is avoided by preservation in formaldehyde (although their sample size was small). We can conclude that the used preservation method for ATOS (immersion for 2 weeks in a 2.7%–4% formaldehyde solution) is effective to inactivate HIV, HBV, HCV, and other reported viruses such as vaccinia. The data reported on betacoronaviruses suggest that it is also the disinfectant of choice for SARS-CoV, although no studies have been published on SARS-CoV-2 itself. The data confirm that temporary storage for at least 2 days at room temperature must be implemented to increase the virucidal effect of formaldehyde. As ATOS are non-life-saving allografts, a strict donor screening protocol in combination with serological screening tests remains essential to guarantee maximal safety for the patient. NAT for the detection of HIV, HBV, and HCV remains necessary to prevent transmission as there is no proof in literature that potentially very high virus loads are inactivated by the preservation method. These data indicate that the used formaldehyde solution provides an extra and very efficient safety layer against the reported viruses in addition to other safety measurements, including NAT. NAT also detects very high virus loads, higher than the loads reported in the literature, for which no evidence of inactivation was found. Virus reduction using formaldehyde solution will definitely close the detection window of donors with low virus load.

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