Oxybuprocaine Induces a False-positive Response in Immunochromatographic SAS Adeno Test

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Objective: To investigate whether a solution of oxybuprocaine hydrochloride, 0.4%, results in a false-positive response in an immunochromatographic SAS Adeno Test.

Design: Experimental study.

Controls: Physiologic saline and 2% lidocaine.

Testing: Each chemical (100 μl) was diluted in a transport medium. Five drops (200 μl) of the resultant solution were dispensed into the round sample well of a test device. Fifteen samples were tested in each group.

Main Outcome Measures: Ten minutes after the start of the test, a colored line in the "specimen" portion of the test membrane was visually read as positive or negative by a masked technician.

Results: No positive reaction was observed in the control groups (physiologic saline and lidocaine). A false-positive reaction was observed in six samples (33.3%) in the oxybuprocaine group. The positive rate was significantly higher in the oxybuprocaine group compared with those in control groups ($P = 0.0062$, Fisher's extract probability test).

Conclusions: Oxybuprocaine may induce a false-positive reaction in an immunochromatographic SAS Adeno Test. We recommend the use of lidocaine, instead of oxybuprocaine, for local anesthesia in taking eye swabs from patients with suspected adenovirus infection. *Ophthalmology 2002;109:808–809 © 2002 by the American Academy of Ophthalmology.*

Ocular adenovirus infection has various clinical manifestations, such as epidemic keratoconjunctivitis (EKC) and pharyngoconjunctival fever. The "gold standard" of the adenoviral diagnosis is an isolating culture of the virus; however, this can take several weeks. A rapid diagnosis of adenoviral infection is necessary to properly treat the patient and to prevent possible nosocomial spread of this highly contagious disease.[1] Recently, physicians have been able to make a rapid diagnosis of ocular adenoviral infection using two different commercial kits; an enzyme immunoassay (ELISA) (Adenoclone, Cambridge BioScience, Worcester, MA)[2–4] and an immunochromatography test (SAS Adeno Test, SA Scientific, San Antonio, TX).[1,4]

We have used SAS Adeno Tests for several years in patients with suspected adenoviral infection. In taking a swab specimen, we routinely use an oxybuprocaine hydrochloride solution (Benoxyl 0.4% solution, Santen, Tokyo, Japan) as a local anesthetic to reduce the patient's pain. Several patients who were diagnosed with bacterial conjunctivitis or allergic conjunctivitis showed positive results in the SAS Adeno Test, and we speculated that local anesthesia with oxybuprocaine may affect the result. Because no information about a possible false-positive reaction to oxybuprocaine was included in the information provided with the SAS Adeno Test (SA Scientific, San Antonio, TX), we investigated whether oxybuprocaine solution could induce a false-positive reaction in the SAS Adeno Test.

Materials and Methods

Three test groups were used for experiments: the oxybuprocaine group (Benoxyl 0.4% solution, Santen), the physiologic saline group (normal NaCl, Otsuka Pharmaceutical, Tokushima, Japan), and the lidocaine group (xylocaine 2% solution, Astra Japan, Osaka, Japan). The physiologic saline and lidocaine groups were used as controls, and the sample number in all three groups was 15. Before the experiments, all the experimental devices and an experimental table were wiped twice with 70% ethanol to prevent possible contamination by adenovirus or other pathogens. The examiners wore sterile gloves and covered their mouths with masks. All the chemicals listed previously and SAS Adeno Test kits (SA Scientific) were unpacked just before the experiment. In each group, 100 μl of the solution was placed on a cotton–wool tip of the kit. The following procedures were performed according to the SAS Adeno Test Procedure Manual. The solution on the cotton–wool tip was mixed with the transport medium (500 μl) in the extraction tube for 5 minutes at room temperature. Five drops (approximately 200 μl) of the resultant solution were dispensed

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The authors have no proprietary interest in any aspect of this study.

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Table 1. False-positive Reaction to an Oxybuprocaine Hydrochloride Solution in SAS Adeno Test

Group	Number	SAS Adeno Test		*P* Value
		Positive	*Negative*	
Oxybuprocaine	15	6	9	0.0062
Physiologic saline	15	0	15	
Lidocaine	15	0	15	

The *P* value was obtained by comparing the oxybuprocaine group and control groups; physiologic saline group and lidocaine group, respectively. Fisher's exact probability test was used for statistics.

into the round sample well of the test device. Ten minutes later, an experienced technician masked to the experimental design, visually read the results. Positive results were defined as the appearance of two colored lines; one in the S area (specimen) and one in the C area (control) of the membrane. Negative results were defined as the appearance of only one line in the C area. Invalid results were defined as no line appearing in the C area even if a colored line appeared in the S area. The number of positive, negative, and invalid results were counted in each group and statistically compared between the groups. Fisher's exact probability test was used for statistics instead of the chi square test, because some probability values were less than 5. Microsoft Excel 98 (Microsoft Corporation, Redmond, WA) was used as the statistical program software.

Results

In the control groups (physiologic saline group and lidocaine group), the results were all negative. In the oxybuprocaine group, 6 of 15 samples (40%) were positive (Table 1). The colored lines in the S area (specimen) were extremely weak compared with those in the C area (control) in all positive samples. The positive rate in the oxybuprocaine group was significantly higher than in the control groups ($P = 0.0062$).

Discussion

The results from this study suggest that oxybuprocaine hydrochloride solution may induce positive reactions in SAS Adeno Tests. The results in the control groups, the physiologic saline group and lidocaine group, were negative, and the rate of positive reactions was significantly higher in the oxybuprocaine group compared with those in control groups. The possibility of contamination by adenovirus was low in this experimental design, and even if adenoviral contamination did occur, it cannot explain the difference in the positive rate between the three groups. Thus, we suggest that the oxybuprocaine hydrochloride solution induced false-positive reactions in the SAS Adeno Test.

According to the SAS Adeno Test Procedure Manual, a positive reaction is defined as the appearance of two colored lines. "One colored line will appear in the S area (specimen) and one in the C area (control). Any colored line in the S area should be considered positive." Colored lines may be lighter or darker than each other. There is no indication that a false-positive reaction may occur in the SAS Adeno Test, and the specificity (true negatives/true negatives + false positives) is reportedly 97.1%.[4] The specificity in this experiment was 66.6% (9/9 + 6), which is markedly lower than that in the previous study.

The manual does not mention anything about the use of local anesthesia in specimen collection from patients. In practice, when a physician collects specimens from the patient's conjunctiva, a marked quantity of conjunctival epithelial cells is required, because the adenovirus locates inside the cells. Without the use of a local anesthetic agent, this procedure would be painful, and we speculate that many physicians use oxybuprocaine hydrochloride solution. Thus, we suggest that special attention should be paid in the interpretation of positive results in the SAS Adeno Test when oxybuprocaine is used. We recommend the use of lidocaine, instead of oxybuprocaine, because in this study lidocaine did not induce a false-positive reaction.

Immunochromatography applied SAS Adeno Test uses monoclonal and polyclonal antibody reagents to selectively detect adenoviral hexon antigen. We did not analyze the mechanism of cross-reaction between the kit and the oxybuprocaine hydrochloride solution in this study. Moreover, because the exact reagent and formula used in the SAS Adeno Test has not been published, further analysis is difficult. It remains unknown what component of the solution, the oxybuprocaine itself, preservatives, or diluting buffer, reacted with the kit, and further evaluation should be performed to investigate the false-positive reaction induced by oxybuprocaine hydrochloride solution in the SAS Adeno Test.

The SAS Adeno Test is very useful for the rapid diagnosis of possible adenoviral conjunctivitis. However, ophthalmologists should be aware of the possibility of a false-positive result when oxybuprocaine hydrochloride solution is used. We recommend the use of lidocaine, instead of oxybuprocaine, because in this study the lidocaine solution did not induce a false-positive reaction.

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