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Virucidal activity against Herpes and Vaccinia virus of 8 antiseptic formulations

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Summary

Results obtained in the evaluation of virucidal activity of 8 commercial antiseptics at their use concentration are reported. As this study, performed according to the method of the Official French Standard (AFNOR), does not involve the 3 viral strains requested by the Standard but only the Vaccinia virus strain and a strain of Herpes virus, the results cannot be expressed in terms of a general virucidal activity and concern exclusively these 2 specific activities. Only one antiseptic (belonging to the oxidant group) is virucidal for both Vaccinia and Herpes virus, while 3 others are effective only for the Herpes virus. The greater susceptibility to antiseptics of the Herpes virus may be caused by the presence of surfactants in some formulations.

Introduction

The AFNOR Standard methods for the evaluation of the virucidal activity of antiseptics and disinfectants (1985, 1986) were published in 1985, but the number of pharmaceutical formulations studied is still limited. As the AFNOR viral strains (Poliovirus, Adenovirus, Poxvirus) are of limited interest in the evaluation of the efficacy of antiseptics, this study deals with only one of these strains (the Vaccinia virus) and with a Herpes virus strain. The experimental procedure (simple dilution) and the interpretation of results are those described in the standard assay NF T 72-180. Antiseptics were studied at their use concentration.

Materials and Methods

Viruses

Vaccinia orthopoxvirus (Desgenettes Hospital, Lyon) and Herpes virus KOS 1 (C.H.U. Toulouse-Rangueil) were grown on Vero cells (ATCC CCL 81). Virucidal assays require viral suspensions containing per ml 10^6–10^9 infection units (I.U.). Viral suspensions, divided in single doses are kept at $-80\,^{\circ}C$.

Antiseptics

Eight antiseptic formulations (of which the bactericidal properties were established elsewhere) (Table 1) were examined. They contain antimicrobial agents belonging to different chemical classes.

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TABLE 1

Commercial antiseptic solutions

Antiseptic (code)	Antimicrobial agent	Manufacturers	Use concentration
A	Polyvidone iodine	Sarget	20%
B	Chlorhexidine	ICI Pharma	2.5‰
C	Carbanilide	Clin-Midy	undiluted
D	Benzalkonium chloride	Pharmascience	undiluted
E	Oxyquinol	Clin-Midy	1/3
F	Mercurial	Labaz	undiluted
G	Trypan blue	L'Arguenon Semb	undiluted
H	Perhydrol (10%)	Gifrer	undiluted

For the assays, the antiseptics were diluted at their use concentration in Hanks solution (Flow 18.104.54).

Method

The simple dilution method is described in the experimental standard assay NF T 72-180 (Afnor, 1985). As indicated in the procedure, 3 preliminary assays must be done to assess the validity of the results:

(1) Determination of the subcytotoxic dilution (higher concentration of the antiseptic allowing no cytotoxic effect on the cells).
(2) Capacity of the cells treated by the antiseptic at the subcytotoxic dilution to develop the viral infection as well as the untreated cells: the titers of the viruses cultivated on treated and untreated cells must be similar (difference 0.5 log).
(3) Determination of the stop-dilution (higher concentration of the antiseptic showing no virucidal activity after abrupt and cold dilution).

The virucidal test

(1) The contact at $30° \pm 1°C$ of the viral suspension with the antiseptic (in the control, the Hanks solution takes place of the antiseptic).
(2) After 15, 30 and 60 minutes of contact, the abrupt cold dilution of the samples as determined in the preliminary assay (stop-dilution).
(3) If necessary, complementary dilution(s) in order to obtain the subcytotoxic dilution are determined in the preliminary assay.
(4) Counts of virus in the samples and in controls are determined according to the standard method using the tables of Wyshak and Detre (1972). The cytopathic effect is observed after 3 days of incubation for the Vaccinia virus and 6–7 days of incubation for the Herpes virus at $37 \pm 1°C$.

The French Standard assay classifies as virucidal the agents which cause, in the standard conditions, a reduction of the 3 viral strains' titres of at least 4 log.

Results

Preliminary assays

Table II reports the subcytotoxic dilutions and the stop-dilutions. In every case, the cells submitted to the subcytotoxic dilution of the different antiseptics, have demonstrated their capacity to develop the viral infection as well as the untreated cells.

Virucidal test

The results are presented in Table III. They are expressed as number of survivors for each formulation and for the two viral strains:

– Perhydrol (H) showed the highest virucidal activity; unfortunately, because of the strong cytotoxicity, the rate of surviving viruses could not be determined accurately.

TABLE 2

Subcytotoxic dilution and stop-dilution

Antiseptic formulation (code)	Concentration tested (%)	Subcytotoxic dilution	Stop-dilution
A	20	10^{-3}	10^{-2}
B	0.5	10^{-3}	10^{-2}
C	90	10^{-3}	10^{-2}
D	90	10^{-3}	10^{-2}
E	30	10^{-3}	10^{-2}
F	90	10^{-3}	10^{-2}
G	90	10^{-2}	10^{-2}
H	90	10^{-4}	10^{-3}

TABLE 3

Log number survivors in the virucidal test

Antiseptic	Concentration (%)	Virus titres in log I.U./ml							
		Vaccinia virus				Herpes virus			
		15	30	60	Control	15	30	60	Control
A	20	5.84	5.54	4.95	7.73	4.95	4.16	< 3	7.16
B	0.5	6.26	5.95	4.46	7.73	4.73	4.26	3.36	7.06
C	90	7.26	7.06	6.06	7.73	5.95	5.16	3.73	6.84
D	90	7.06	6.84	5.26	7.54	5.06	4.73	< 3	6.35
E	30	7.44	7.26	6.16	7.84	6.44	6.06	3.54	7.16
F	90	6.44	5.95	4.84	7.73	4.54	3.06	< 3	7.06
G	90	6.73	6.46	5.95	7.54	5.73	4.95	3.44	7.16
H	90	< 4	< 4	< 4	7.84	< 4	< 4	< 4	7.26

The numbers 15, 30 and 60 are the contact times (min) for assays; the contact time for the control is 60 min.

- Formulation (B) had a slight virucidal capacity for the two viruses: the reduction rate was between 3 and 4 log for the Herpes virus according to the contact time.
- For 3 formulations virucidal efficacy observed towards the herpetic strain was: F was effective in 30 min, A and D in 60 min.
- The 3 last preparations (C, E and G) were slightly effective in 60 min on the herpetic strain.

Discussion

According to the French Standard, a virucidal formulation should reduce by 4, the log number of the 3 reference-viral suspensions (two uncoated virus strains: Poliovirus and Adenovirus, and one coated virus strain: Vaccinia virus). Our study did not allow us to establish whether the tested antiseptics were virucidal according to this standard; but it demonstrated whether these antiseptics, at their common use concentration, are virucidal for the Vaccinia virus and the Herpes virus, by using a standard methodology including all the preliminary tests needed for the assessment of a real virucidal effect.

Among the 8 antiseptics studied, some formulations (A, B, E, H) are bactericidal (Chanal et al., 1978; Daval, 1978) according to the French Standards (AFNOR, 1985; French Pharmacopea, 1987). Our results show that there is no correlation between the bactericidal and the virucidal activities of these formulations.

According to Klein and Deforest (1983) the lipophile-coated virus is more susceptible to the virucidal agents than the uncoated virus. Though the two viruses studied here possess coats, we have observed noticeable differences in their susceptibility to the studied antiseptics, the Vaccinia virus being more resistant than the Herpes virus: perhydrol (H) only is active on the Vaccinia virus whereas the 7 other formulations demonstrate more or less efficacy on the Herpes virus. The susceptibility of the Herpes virus is observed with antiseptics (D–F) containing antimicrobial agents known as not virucidal (Damery, 1986; Damery and Cremieux, 1986). This phenomenon could be explained in some formulations (particularly in the formulation F) by the presence of surfactants able to modify the organization of the coat in the herpetic virion; in the same conditions the structure of the Vaccinia virus remains unaltered. Besides it has been shown that the excipient can cause a reduction of the virucidal activity: the concentration of iodine necessary to reach the virucidal effect must be increased when iodine is fixed on a support as P.V.P. (AFNOR, 1982; Damery, 1986).

Concerning the methodology we have noticed that the simple dilution technique is rather difficult to apply to some antiseptics which are cytotoxic (results not shown). To suppress the cytotoxicity, an important dilution rate is needed in

the preliminary assays; consequently, the virucidal test requires the use of viral suspensions with high titer in order to calculate the count of survivors which is performed at the end of the test at the subcytotoxic dilution. Two other techniques have been standardized in the French Standard: one, founded on gel-filtration, is not satisfactory for some formulations (for example with P.V.P. iodine); the second, simple dilution followed by virus concentration by ultrafiltration, is difficult to perform and more expensive. Modification of this latter technique would be suitable to determine the antiviral activity of cytotoxic formulations.

Conclusion

The methodology of the Simple Dilution Technique as described by the AFNOR has been applied to determine the virucidal activity of 8 antiseptics towards the Vaccinia virus and the Herpes virus. If in some cases the virucidal activity, when observed towards the two viruses, may be linked to the antimicrobial drug, the role of surfactants in the excipient must be taken into account when the Herpes virus alone is susceptible.

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