

Influence of normal human serum on the determination of trichomonocidal drug concentrations

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Agglutinating and complement-fixing antibodies to *Trichomonas vaginalis* have been found in sera from infected patients of both sexes and also in control sera (Trussell and Wilson, 1942; Tatsuki, 1957). Weld and Kean (1958) described a cytolytic factor in human serum capable of destroying *T. vaginalis* in a few minutes. This factor was inactivated by heating for 30 min. at 56°C. and lost activity on storage. All the human sera examined, as well as sera from rabbits, hamsters, guinea-pigs, rats, and dogs, were deleterious to *T. vaginalis*. This paper confirms the above report on the cytolytic factor. The influence of serum on determinations of the concentration of trichomonocidal drugs in serum has been investigated.

Material and methods

STRAINS

Ten *Trichomonas vaginalis* strains isolated from clinical material and a reference strain, *T. vaginalis* Milan, kindly supplied by Dr. I de Carneri, were used.

CULTIVATION TECHNIQUE

The trichomonocidal effects of metronidazole, nitrimidazine, and serum were tested, using doubling dilutions in serum, saline, or thioglycolate medium in a volume of 1 ml. per test-tube. 1 ml. of a suspension in thioglycolate medium of 8×10^4 *T. vaginalis* organisms per ml. was added to each of the test-tubes, giving a final concentration of 4×10^4 viable organisms per ml. After 3 days' incubation at 37°C., 0.2 ml. from each test-tube was transferred to 4 ml. Diamond's medium (Diamond, 1957) and the subculture was incubated for 5 days. Evaluation of trichomonocidal activity was based on the final result of subculture.

Results

As shown in the Table, all concentrations of nitrimidazine were completely trichomonocidal in fresh human serum diluted 1/2, 1/4, and 1/8, when incubated with *Trichomonas vaginalis*, strain Milan, in a concentration of 40,000 organisms/ml. for 3 days. No viable *Trichomonas vaginalis* could be detected by subculture in Diamond's medium. However, low

concentrations of nitrimidazine in serum diluted 1/16 permitted the organisms to survive. When nitrimidazine was dissolved in serum diluted 1/32 or more, *Trichomonas vaginalis* survived in the same concentrations of nitrimidazine as when the drug was dissolved in saline or thioglycolate medium. The lowest trichomonocidal concentration of nitrimidazine in serum inactivated for 30 min. at 56°C. was the same as for nitrimidazine in saline or in serum diluted 1/32 or more.

TABLE

Nitrimidazine dissolved in	Concentration of nitrimidazine ($\mu\text{g./ml.}$)					
	2.5	1.25	0.6	0.3	0.15	0.08
Human serum						
1:2	-	-	-	-	-	-
1:4	-	-	-	-	-	-
1:8	-	-	-	-	-	-
1:16	-	-	-	-	+	+
1:32	-	-	-	+	+	+
1:64	-	-	-	+	+	+
1:128	-	-	-	+	+	+
1:256	-	-	-	+	+	+
Inactivated (36°C., 30 min.)	-	-	-	+	+	+
Saline	-	-	-	+	+	+
Thioglycolate medium	-	-	-	+	+	+

Trichomonas vaginalis, Milan, incubated for 3 days in a series of concentrations of nitrimidazine, each present in a series of dilutions of human serum. (-) indicates a trichomonocidal effect; no living organisms could be detected by subculture. (+) indicates that living organisms were detected by subculture.

Reduced and varying trichomonocidal effects were obtained when serum that had been stored for some days at +4°C. or -20°C. or serum that had been frozen and thawed several times was used.

Results similar to those shown in the table were obtained when the sensitivity of *Trichomonas vaginalis* to metronidazole in serum was tested. Of the strains investigated, *Trichomonas vaginalis* Milan was the most sensitive to the effect of human serum. Different strains of *Trichomonas vaginalis* isolated in the routine laboratory showed different sensitivities to human serum. For three of our ten strains, fresh human serum diluted 1/2 had no detectable trichomonocidal effect with the method used.

Discussion

The chemotherapy of *Trichomonas* was significantly improved by the discovery of metronidazole (Cosar, Julou, and Bénazet, 1959). Nitrimidazine has been stated to have *in vitro* activity of the same order as that of metronidazole (de Carneri, 1969). The absorption of metronidazole and nitrimidazine after oral administration has been investigated by polarography (Kane, McFadzean, Squires, King, and Nicol, 1961) and gas-chromatography (Giraldi, Tosolini, Dradi, Nannini, Longo, Meinardi, Monti, and de Carneri, 1971). However, reports on serum levels of metronidazole (Jennison, Stenton, and Watt, 1961) and nitrimidazine (de Carneri, 1969), as determined by a biological method, have been presented. By that method, serum containing the drug under investigation is diluted and then inoculated with a strain of *Trichomonas vaginalis*.

The experiments presented in this paper show that fresh normal human serum in dilutions up to 1/8 has a trichomonocidal effect under the conditions used for determination of the serum level of trichomonocidal drugs. The consequence of this is that, when the serum level of metronidazole or nitrimidazine is low or moderate and the serum is diluted 1/16 or less, the trichomonocidal effect of the serum may be confused with that of the drugs, when biological determinations of drug concentrations in serum are performed, so that falsely high results can be obtained. The experiments also show that this can be avoided by inactivating the serum at 56°C. for 30 minutes. The clinical importance of the differences in sensitivity to human serum among *Trichomonas* strains reported in this paper is unknown.

Summary

Fresh normal human serum has a trichomonocidal effect which can interfere with biological determinations of serum levels of metronidazole and nitrimidazine. When investigating heat-stable trichomonocidal drugs this can be avoided by inactivating the serum at 56°C. for 30 minutes. *Trichomonas* strains were found to differ in their sensitivity to serum.

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L'influence de sérum humain normal dans l'étude des médicaments trichomonocides

SOMMAIRE

Le sérum humain frais normal a une action trichomonocide qui peut intervenir dans la détermination biologique des taux sériques de métronidazole et de nitrimidazine. Lorsque l'on étudie des médicaments dont l'action trichomonocide est thermostable, cet inconvénient peut être évité en inactivant le sérum à 56°C. pendant 20 minutes. Les souches de trichomonas ont manifesté des différentes sensibilités vis-à-vis du sérum.