A SIMPLE URINE TEST FOR SULFONAMIDES

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The lack of a simple, reliable urine assay for sulfonamides has led to difficulties in the interpretation of in vitro and in vivo tests for the assessment of drug sensitivity of Plasmodium falciparum in patients who had received unknown medication before the test. There is a simple urine assay for 4-aminoquinolines (Leljveld & Kortmann, 1970), but the increasing use of sulfonamides, usually in combination with pyrimethamine, necessitates also a test for sulfonamides which is applicable in the field.

Feigl & Anger (1975) described a modification of the reliable Bratton-Marshall reaction for sulfonamides and produced a very sensitive and simple spot test on filter-paper. The reagent is, however, neither readily available nor listed in the current chemical catalogues.

Based on the Bratton-Marshall technique and on a suggestion by Wehrli (1969), we have developed a urine test for sulfonamides which can also be carried out in simple, peripheral laboratories.

Reagents

(1) Sodium nitrite solution; 0.2% in distilled water.

(2) Hydrochloric acid, concentrated.

(3) Bratton-Marshall solution (BM); dissolve 20 mg N-(1-naphthyl)-ethylenediamine dihydrochloride in 20 ml distilled water, adding three drops of concentrated hydrochloric acid.

Procedure

(1) Pipette 1 ml of urine into a test-tube.

(2) Add one drop of sodium nitrite solution (reagent 1) and two drops of concentrated hydrochloric acid (reagent 2) and mix. Let it stand for one minute.

(3) Add three drops of BM (reagent 3) and mix.

Evaluation

(1) The test is positive for free sulfonamides (and other diazotisable arylamines) when the liquid takes on a persistent purple colour.

(2) The test is negative if the liquid does not take on a purple colour or if the purple colour quickly disappears and the liquid turns a greenish or brownish colour.
Comments

(1) The test gives reliable results and is quite sensitive down to a sulfonamide concentration of 5 mg/l urine.

(2) The lower the sulfonamide content, the longer it takes the colour reaction to develop (30 seconds to 30 minutes).

(3) Urinary sulfadoxine concentrations one week after a normal treatment dose (1.5 g) were found to be of the order of 15 mg/l (Haegi, 1966); thus the test should prove to be suitable for the intended purpose.

(4) The suggestion made by Wehrli (1969) was that the use of sulfamate to destroy excess nitrous acid between steps (2) and (3) of the test procedure could be overlooked. The destruction of excess nitrous acid is, in fact, absolutely indispensable, but it is taken care of by urea, which reacts with nitrous acid in a similar manner and which occurs in urine in proportions corresponding to many times the usually employed amounts of sulfamate. This phenomenon has been ascertained by laboratory trials and moreover by a one-year field use of the test, as reported by Souza (1981).

(5) Drugs other than sulfonamides susceptible of giving a positive Bratton-Marshall reaction are: nitrazepam, clonazepam, flunitrazepam and substances that carry an aromatic primary amino group or substances that are metabolized to such compounds.

(6) Reagents (1) and (2) are quite stable at room temperature. Reagent (3), kept at 5-10°C in a brown bottle, is stable for at least two months.

RESUME

L'absence d'une méthode simple et fiable de recherche des sulfamides dans l'urine a entraîné des difficultés dans l'interprétation des épreuves in vitro et in vivo d'évaluation de la sensibilité de Plasmodium falciparum chez des malades ayant reçu un traitement médicamenteux inconnu avant l'épreuve. Une épreuve urinaire basée sur la technique de Bratton-Marshall et sur une suggestion de Wehrli, a été mise au point pour les sulfamides. Cette épreuve exige trois réactifs : une solution de nitrite de sodium (à 0,2 % dans l'eau distillée); de l'acide chlorhydrique concentré; une solution de Bratton-Marshall. Elle est simple à réaliser et à évaluer et peut être effectuée dans les laboratoires périphériques. Elle donne des résultats fiables et est tout à fait sensible jusqu'à une concentration urinaire de sulfamides de 5 mg/l. Les médicaments autres que les sulfamides susceptibles de donner une réaction de Bratton-Marshall positive sont le nitrazépam, le clonazépam, le flunitrazépam et des substances portant un groupement amine aromatique primaire, ou des substances métabolisées en de tels composés.

REFERENCES