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Chemical behaviour of noxythiolin in biological fluids

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Summary

A variety of different analytical methods were employed to study the chemical behaviour of noxythiolin and its degradation products, *N*-methylthiourea and formaldehyde, in biological fluids. It was demonstrated that, although the presence of urine had little adverse effect on the stability of noxythiolin, the formaldehyde released was bound by urine components. Although both whole serum and some serum dilutions markedly increased the rate of noxythiolin degradation in vitro, substantial amounts of the released formaldehyde were bound by serum constituents as determined by direct formaldehyde analysis using differential pulse polarography. Confirmation of this binding was provided by colorimetric estimation of available formaldehyde present in equilibrated noxythiolin/urine solutions where approximately 45% of the released formaldehyde was bound. The results presented might well explain the lack of toxicity associated with noxythiolin in clinical use.

Introduction

Noxythiolin (*N*-methyl-*N'*-hydroxymethylthiourea) is an effective antimicrobial agent which is used extensively in clinical practice for the prevention and eradication of infection from the body surface and specified cavities, particularly for urological and intraperitoneal use (Williams et al., 1976; Harper, 1981; Antos, 1980). The compound, which also has anti-adherent properties, is available as a dry powder and is reconstituted as aqueous solutions 1% w/v for prophylactic and 2.5% w/v for therapeutic use.

Although the degradation pattern of noxythiolin in aqueous solutions has been well documented (Irwin et al., 1984; McCafferty et al., 1984) there is little information available on the chemical behaviour of the compound in biological fluids. The effect of the latter on the degradation pattern of noxythiolin is considered to be of some importance since formaldehyde is a degradation product of the compound. Therefore, since noxythiolin is widely used in the presence of biological fluids, for example urine, the effect of these on the chemical behaviour of the compound was examined using a number of different analytical techniques.

Materials and Methods

Chemicals

Noxythiolin was supplied as Noxyflex S by Geistlich Sons Ltd., Chester, U.K. All other chemicals used were of Analar or equivalent quality.

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Biological fluids

Fresh whole human blood was obtained as required from the Northern Ireland Blood Bank, Belfast. Serum was obtained by centrifuging blood samples at 3000 rpm for 10 min after which the supernatant was collected. Sterile deioinised water was used to prepare 20% v/v serum. Preparation of protein-free serum involved vortexing acetonitrile with serum (2:1 ratio) for 30 s, centrifuging at 3000 rpm for 5 min, then carefully collecting the supernatant. Fresh urine was obtained from healthy male volunteers and membrane-sterilised if required. Fluid dilutions were made with sterile deionised water.

HPLC analysis

The HPLC method previously described was employed, with slight modification, in the present study to monitor the concentration of noxythiolin and *N*-methylthiourea (McCafferty et al., 1984). In order to protect the HPLC column, proteins were precipitated from serum samples before the appropriately diluted samples were introduced onto the column. Precipitation was achieved by adding 5 ml of the sample to 10 ml of ice-cold acetonitrile, vortexing thoroughly for 30 s, then centrifuging at 3000 rpm for 3 min at 2°C. The presence of acetonitrile did not affect noxythiolin stability.

Polarographic analysis

The concentration of formaldehyde in noxythiolin solutions was determined using a differential pulse polarographic method as previously reported by us (Woolfson et al., 1985).

Colorimetric analysis

The method of Nash (1953) was also used to monitor formaldehyde concentrations in noxythiolin solutions (McCafferty et al., 1984).

Preparation of noxythiolin containing fluids

Fresh noxythiolin solutions (2.0% w/v) were prepared using, as appropriate, 20% v/v urine, 20, 100% v/v serum, 20% v/v protein-free serum and incubated at 37°C. Samples of these solutions were analysed at various time intervals for the presence of noxythiolin and its degradation products.

Results and Discussion

When noxythiolin is instilled into the bladder of a patient it can be assumed that a residual volume of urine is present (Stickler et al., 1981). Furthermore, urine is continuously flowing into the bladder from the ureters, thereby further reducing the initial concentration of noxythiolin. In order to mimic these effects in-vitro under controlled conditions a standard 2% w/v solution of noxythiolin was prepared in 20% v/v urine. It can be seen from Fig. 1a that the degradation of noxythiolin in urine at 37°C is only slightly increased when compared to control (noxythiolin in water only). This is confirmed by Fig. 1b which shows the release of *N*-methylthiourea, a degradation product of noxythiolin, under the same conditions. In contrast, no formaldehyde could be detected polarographically during this incubation period. Although noxythiolin degradation can be represented as a first order forward reaction being opposed by a second order reverse reaction (McCafferty et al., 1984) the initial rate of degradation can be treated as apparent first order for comparatively small amounts of degradation. The apparent first order rate constants for the degradation of noxythiolin that were derived from analysis of noxythiolin as well as its degradation products are shown in Table 1. It can be seen that the apparent first order degradation rate constants obtained for noxythiolin in aqueous solution are similar irrespective of whether these were obtained from analysis of noxythiolin (by HPLC) or from either *N*-methylthiourea (by HPLC) or formaldehyde (by polarography). However, the overall chemical behaviour of noxythiolin and its degradation products would appear to be affected by the presence of 20% v/v urine (Table 1). Although the rate of degradation of noxythiolin is only slightly increased, formaldehyde could not be detected in these solutions under the experimental conditions employed. Since there was little interference in the analytical method as judged from spiked samples it seemed likely that the formaldehyde generated from noxythiolin was being rapidly bound by urine components. Unfortunately colorimetric analysis was unsuitable to detect the presence of formaldehyde in these solu-

tions due to the prolonged time (5 h) required for colour development in comparison to the relatively short (160 min) incubation period. However, when this analytical technique was used for samples incubated for longer time periods (Fig. 2) it can be seen that the equilibrium concentration of formaldehyde was low compared to control. The apparent equilibrium concentration of formaldehyde released from a 2% w/v noxythiolin containing 20% v/v urine incubated at 37 °C for a 6-day

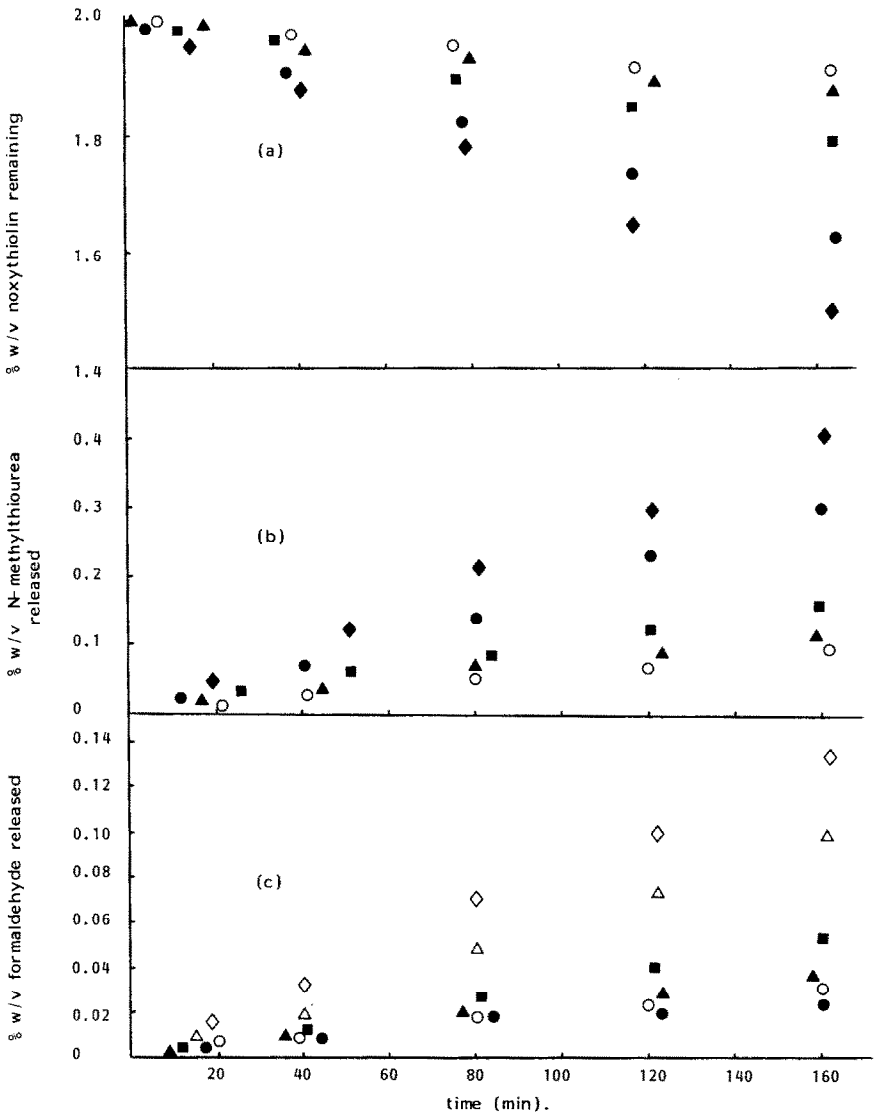

Fig. 1. Degradation of noxythiolin and release of *N*-methylthiourea and formaldehyde in various fluids at 37 °C. a: degradation of 2% w/v noxythiolin in water (○); 20% v/v urine (▲); 20% v/v protein-free serum (■); 20% v/v serum (●); 100% v/v serum (◆). b: release of *N*-methylthiourea from 2% w/v noxythiolin in water (○); 20% v/v urine (▲); 20% protein-free serum (■); 20% v/v serum (●); 100% v/v serum (◆). c: release of formaldehyde from 2% noxythiolin, directly determined by polarography, in water (○); 20% v/v serum (●); estimated indirectly from HPLC data, in: 20% v/v urine (▲); 20% v/v protein-free serum (■); 20% v/v serum (△); 100% v/v serum (◇).

TABLE 1

Apparent first order rate constants ($k_1 \cdot 10^{-2}$) for noxythiolin degradation at 37 °C in various fluids estimated from noxythiolin and its degradation products

	Water		20% v/v Urine		20% v/v Serum		100% v/v Serum		20% v/v Protein-free serum	
	k_1	r	k_1	r	k_1	r	k_1	r	k_1	r
Noxythiolin	2.46 ± 0.23 *	0.97	2.68 ± 0.28	0.97	8.19 ± 0.35	0.99	11.80 ± 0.45	0.98	3.74 ± 0.31	0.97
N-Methyl-thiourea	2.76 ± 0.14	0.99	3.17 ± 0.09	0.99	8.24 ± 0.17	0.99	11.99 ± 0.32	0.99	4.17 ± 0.19	0.98
Formalde-hyde	2.88 ± 0.12	0.99	0.00 [a]		1.83 ± 0.05	0.99	N.D. [b]		0.00 [a]	

[a] No detectable formaldehyde
[b] Not determined
* Mean data ± S.D.

period was 0.16 ± 0.007% (S.D.) w/v whereas the value found in the control was 0.35 ± 0.016% (S.D.) w/v. This corresponds to approximately 45% of the available formaldehyde being bound. Preliminary experimentation indicated that components present in the urine samples did not interfere with the colorimetric assay. Therefore, both the polarographic and colorimetric estimation of formaldehyde in urine containing noxythiolin indicate that formaldehyde is bound by urine components. It has been reported that in neat urine approximately 85% of the available formaldehyde present in noxythiolin solutions can be chemically bound to urine components (Kingston, 1965). Furthermore, Jones and Mashford (1983) have reported the presence of *N*-hydroxymethylurea and *N,N'*-bis(hydroxymethyl)thiourea in the urine of Sprague–Dawley rats following treatment with noxythiolin. The former two compounds are urea–formaldehyde adducts. Interestingly, neither noxythiolin nor *N*-methylthiourea were bound by the urine constituents (Fig. 1, Table 1). This can be interpreted by the fact that the disappearance of noxythiolin was closely matched by the release of *N*-methylthiourea (confirmed by the similar rate constants illustrated in Table 1). It is possible that the slight increase in noxythiolin degradation observed in urine (Table 1) could be attributed to the effective removal of formaldehyde from solution which would tend to promote the forward (degradation) reaction.

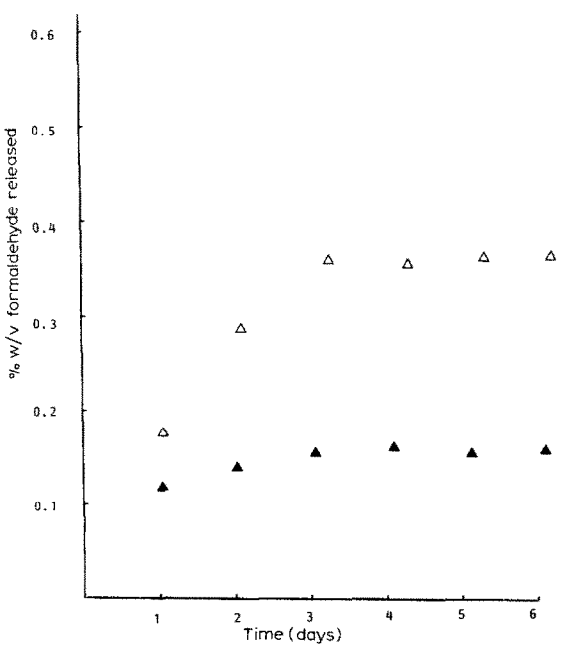

Fig. 2. Release of formaldehyde (determined colorimetrically) from 2% w/v noxythiolin incubated at 37 °C in water (△); 20% v/v urine (▲).

Noxythiolin is also recommended for use intraperitoneally where it will come into contact with blood filtrates. Furthermore, when absorbed from the peritoneal cavity, the compound will come into contact with serum (Rosenfeldt et al., 1981). In an attempt to mimic these conditions in vitro a series of different drug/fluid combinations were prepared and the chemical behaviour of

noxythiolin and its degradation products were monitored over a 160-min period (Fig. 1). As can be seen from Fig. 1a and Table 1 there was only a slight increase in the rate of noxythiolin degradation in the presence of 20% v/v protein-free serum whereas degradation was markedly increased with both 20 and 100% v/v serum. These results were confirmed by following the appearance of *N*-methylthiourea in the solutions (Fig. 1b). For example, the rate of noxythiolin degradation in whole serum was approximately 5 × greater than the corresponding water control (Table 1). In contrast, the presence of 20% v/v protein-free serum only increased the degradation rate by a factor of about 1.5. The degradation of noxythiolin in these fluids was closely mirrored by the release of *N*-methylthiourea as can be seen from the similar apparent first order rate constants obtained from the two compounds (Table 1).

The increased degradation of noxythiolin, particularly in serum fluids, can be explained by the presence of non-specific enzymes in fresh serum which can catalyse noxythiolin degradation (Mashford and Jones, 1982). In contrast, when most of these enzymatic systems were precipitated from solution (protein-free serum) then the rate of noxythiolin degradation was substantially reduced (Fig. 1, Table 1). Although noxythyiolin stability was adversely affected in the presence of 20% v/v serum the apparent release of formaldehyde (Fig. 1c) and the apparent first order rate constant derived from this release (Table 1) were reduced even when compared to water controls. Examination of Fig. 1c and Table 1 suggests that substantially more formaldehyde (by approximately a factor of 5) should have been released from this solution than is apparent from the analytical data based on direct formaldehyde determination. None of the latter could be detected in the protein-free sample. It seems likely that formaldehyde was being bound by the constituents of these fluids such as serum proteins or smaller molecules, e.g. cysteine and urea (Mashford and Jones, 1982).

Although it has been recently reported that noxythiolin is rapidly degraded in the presence of urine in vivo to yield relatively large amounts of formaldehyde, the latter was estimated indirectly (Cottrell and Fitzpatrick, 1986). The present study has indicated that not only did urine appear to have little effect on the stability of noxythiolin but that any formaldehyde released is likely to be chemically bound, probably in the form of urea–formaldehyde adducts. Clearly, this type of binding might explain the lack of toxicity associated with the clinical use of noxythiolin.

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