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Lack of mutagenicity of nifedipine: a possible metabolic implication

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Summary

Nifedipine, being a nitro aromatic compound, is capable of undergoing reduction via hydroxylamino intermediate to an amine. Such an intermediate is a mutagenic culprit. The urinary metabolites of nifedipine were investigated in order to allay the fear of the existence of this metabolic route in humans. Nifedipine (20 mg) was administered twice daily for 2 weeks. No nitro-reduction product was detected over a 1-month period. Nifedipine lacks mutagenicity in the absence or presence of drug metabolizing microsomes in *Salmonella typhimurium* TA 98 and *Salmonella typhimurium* TA 100.

Résumé

La nifédipine est un composé nitro aromatisé, qui se réduit à une amine en passant par l'aminohydroxyl, un produit mutagénique comme intermédiaire. Pour déterminer s'il existe une telle route métabolique chez l'homme les métabolites urinaires de nifédipine ont été analysés. Une dose à 20 mg de nifédipine deux fois par jour pour deux semaines a été administrée. Il n'y avait pas eu de nitro réduction pendant un mois d'observation. La nifédipine est dépourvue de toute propriété mutagénique en l'absence ou en présence de microsomes métabolisant chez la *Salmonella typhimurium* TA 98 et la *Salmonella typhimurium* TA 100.

Introduction

Nifedipine is a calcium antagonist used in the management of systemic hypertension [1]. It acts primarily by inhibiting the influx of extracellular calcium into smooth muscles and cardiac muscles through slow calcium channels. Apart from exhibiting such calcium entry blockade, nifedipine is capable of sequestrating calcium ions; in other words, nifedipine may deplete a calcium pool by mere complexation procedures.

Numerous drugs containing the aryl nitro group are known to be mutagenic [2–4]. Some nitro aromatic compounds require activation by nitroreductases for mutagenic activity [4,5]. The reduction of the nitro functionality of these compounds gives active hydroxylamino intermediates, which are possible causative mutagenic agents.

Nifedipine is highly absorbed from the gastrointestinal tract after sublingual, oral and rectal administration. The bioavailability of nifedipine is about 56–77% due to pre-systemic metabolism [6]. Nifedipine is almost completely metabolized in the body. About 80% of absorbed dose is excreted through the kidneys in the form of highly water-soluble metabolites (Fig. 1). The objective of this project was to investigate the human urine and explore the possibility of retrieving the reduction product of nifedipine. If such a metabolic biotransformation occurs, taking into consideration the average length of time subjects are kept on nifedipine antihypertensive therapy, it would be appropriate to highlight the potential mutagenic danger that might occur via the metabolic reduction route. We, therefore, investigated the urinary metabolites in Nigerian subjects and the mutagenicity of nifedipine in the presence and absence of rat liver microsomes and required contactors.

Patients and methods

Ultraviolet spectra were recorded using Pye-Unicam SP8–400 UV spectrophotometer

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(Cambridge, U.K.). Thin layer chromatography (TLC) was carried out on silica gel GF_{254} mesh 60 (BDH), the solvent system was ethyl acetate : methanol : water (7:3:1). These solvents were of analytical reagent grade from Aldrich Chemical Company (Milwaukee, U.S.A.). The extraction solvents were ethylacetate and chloroform (analytical grade, Aldrich). Nifedipine, the reference substance, and Adalat (nifedipine, 20 mg) tablets were the generous gift of Bayer (Nig.) Limited Nigeria. Dragendoff reagent was freshly prepared according to the British Pharmacopoeia method.

Nitro-reduction of nifedipine was effected by adding about 20 mg nifedipine to a mixture of Zn/HCl/MeOH and refluxing for 30 min, followed by filtration. The filtrate obtained from the mixture was extracted into ethyl acetate. 2,6-Dimethyl-4 - (nitrophenyl)-1,4-dihydropytidine-3-carboxyl-5-carbomethoxylate, M-1 (Fig. 1) was obtained from mild hydrolysis of nifedipine using 2% HCl (in methanol), and recrystallized from chloroform. M-2′ is a metabolite extracted from urine. It has an ultraviolet spectrum with two maxima λ max 220–240 nm and 310–330 nm. The latter absorbance could be due to a lactone group and as such M-2′ may be identical with M-2 in Fig.1.

Six healthy male volunteers participated in this study. They had not been treated with any drug for 1–2 months prior to the start of study. One tablet of nifedipine (20 mg) was administered to the subjects twice daily for a period of 14 days. Urine samples were collected starting from day 2, and thereafter every other day over a period of 4 weeks. Urine samples were then extracted with ethyl acetate, and co-chromatographed on silica gel GF_{254} plates (0.5 mm) with authentic nifedipine, M-1, M-2′ and nitro-reduction product, M-3, using the solvent system ethyl acetate : methanol : water (7:3:1). Thin layer chromatograms were examined under a UV lamp and by spraying the plates with Dragendorff reagent.

The *Salmonella* mutagenicity assay employed

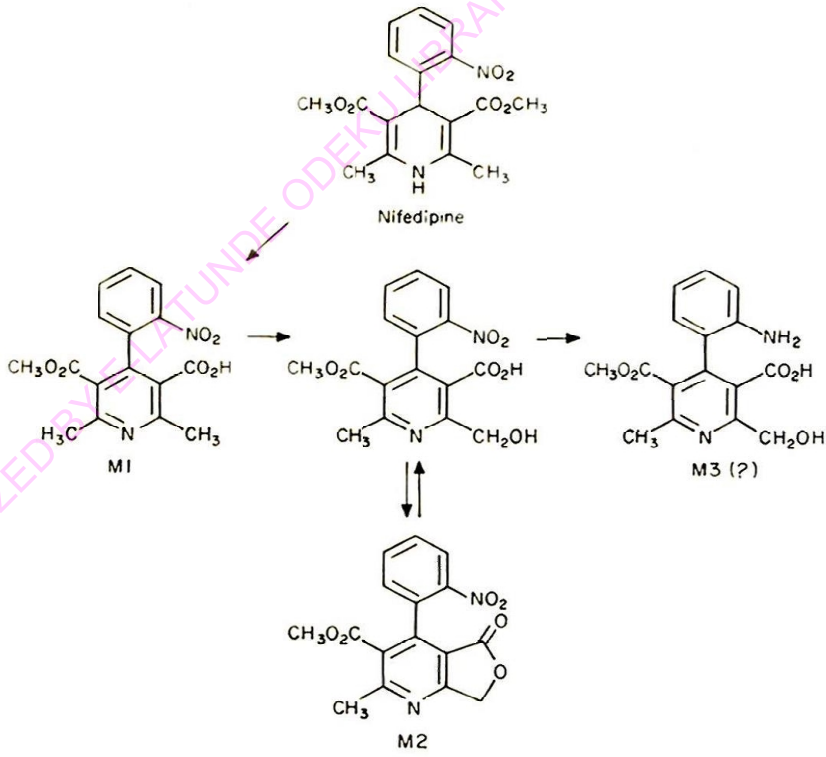

Fig. 1. Nifedipine metabolism.

in this study was that developed and described by Ames, Lee and Durston [7]. The indicator micro-organism (histidine auxotrophs), test agent (nifedipine) and, when indicated, the rat liver preparation (including the required co-factors) were incorporated into the overlay. These mixtures were incubated at 37°C in the dark for 2 days and revertants (mutants) to histidine-independence were enumerated. Liver microsomes (the S-9 post-mitochondrial fraction) were prepared from Sprague-Dawley rats.

Results and discussion

Thin layer chromatographic analysis (Fig. 2) shows that within the first 2 weeks of drug administration, three spots were detected from urine extract. Two of the spots had identical R_f values with nifedipine and M-1 (Fig. 1). Ultraviolet studies of the third spot showed a maximum absorption band of 310-340 nm, which is ascribable to the lactone group. This spot probably corresponds to M-2 (Fig. 1). By the end of the third week, drug intake having stopped in the previous week, analysis showed two prominent spots corresponding to metabolites, M-1 and probably M-2. The urine samples obtained during the fourth week showed a weak and single spot, corresponding probably to M-2.

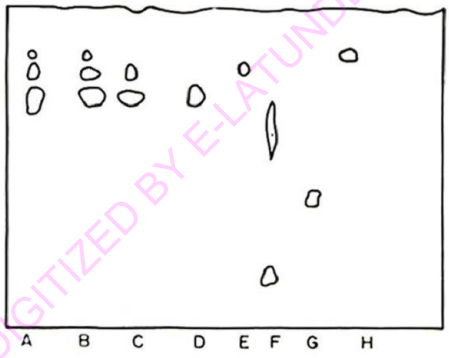

Fig. 2. Thin layer chromatogram of nifedipine and its metabolites. (A) Metabolites extracted from week-1 urine, (B) metabolites extracted from week-2 urine, (C) metabolites extracted from week-3 urine, (D) metabolites extracted from week-4 urine, (E) mild hydrolysis product of nifedipine, (F) prolonged hydrolysis product, (G) nitro-reduction product, (H) nifedipine.

The plasma half-lives of M-1 and M-2 have been shown to be much greater than that of nifedipine, according to Raemesch and Sommer [6]. M-1 is excreted with a half-life of approximately 10 h, M-2 is known to undergo kidney tubular reabsorption and this lends support to its longer half-life of 4–5 days. A cummulative effect of M-2 is thus expected during multiple dosing. A comparison of all the ultraviolet spectra of the extracted urinary metabolites and chromatographic R_f values of authentic samples (Fig. 2) excluded the possible existence of a nitro-reduction metabolite, M-3 in the human urine.

A crude dose–response plot, as constructed by the computer program 'Ames Fit', which is simply the best straight line fit of dose versus number of revertant per plate (and not a true dose–response plot), showed no significant trend or intense response, implying that the concentration range of nifedipine employed in this study was neither lethal nor mutagenic to the strains employed. Mutation ratio relative to control was computed; values less than two for the strains tested were obtained in the presence and absence of microsomes and required co-factors, indicating that nifedipine is not mutagenic in these strains. Figures 3–6 show computer curve fitting of mutation rate versus moles of nifedipine using *Salmonella typhimurium* TA 98 and TA 100 in the presence or absence of microsomes. The curves were obtained with curve Fitter software (a product of Interactive Microwave Inc.) by using the cubic spline interpolation method.

There are a number of reports that metronidazole and other nitro-heterocyclic substances can be reduced by rat liver enzymes; the enzymes implicated have been xanthine oxidase, NADPH-cytochrome C reductase and aldehyde oxidase [7–14]. In some cases, the isolation of the hydroxylamino derivative has been reported [8,12]. In other cases, although only the amino derivative was recovered, the hydroxylamino intermediate was implicated [11,13,14]. The hydroxylamino analogues of nitrofurantoin and metronidazole have been implicated in the mutagenicity of these nitro aromatic drugs [5,15]. Our findings indicate that nifedipine, a nitro aromatic drug, is not metabolized to an amine in humans. This is probably due to the fact that the nitro group in nifedipine (Fig. 1) is strategically placed, and the steric hindrance

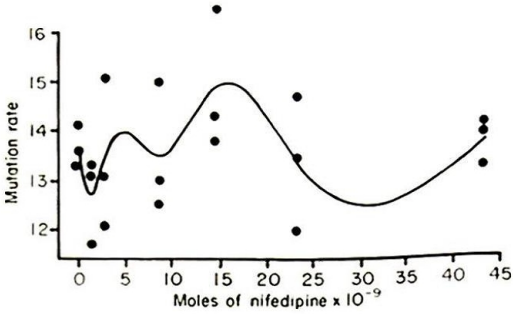

Fig. 3. Mutation rate of *S. typhimurium* TA 98 versus moles of nifedipine in the absence of microsomes.

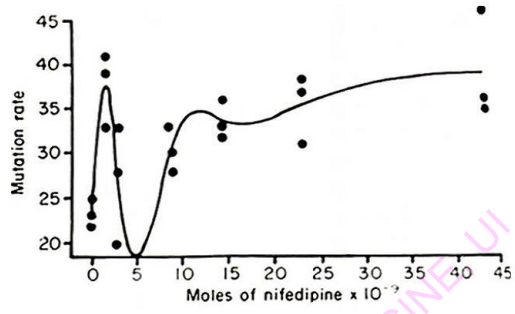

Fig. 4. Mutation rate of *S. typhimurium* TA 98 versus moles of nifedipine in the presence of microsomes

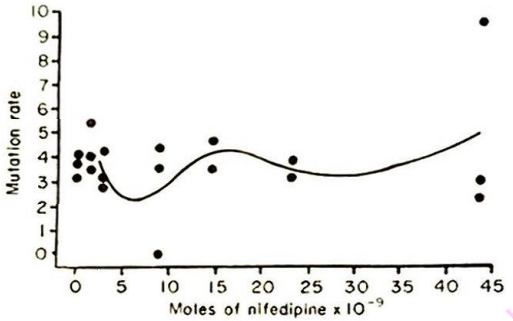

Fig. 5. Mutation rate of *S. typhimurium* TA 100 versus moles of nifedipine in the absence of microsomes.

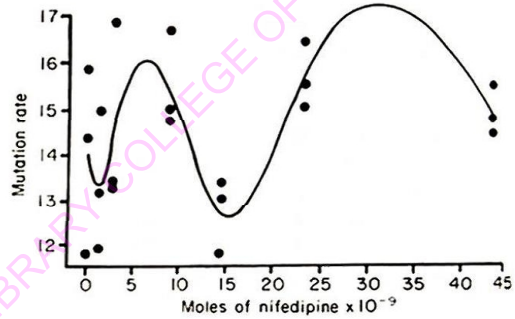

Fig. 6. Mutation rate of *S. typhimurium* TA 100 versus moles of nifedipine in the presence of microsomes.

due to the proximate groups debar or slow down the effect of any approaching reductase enzyme. It is also not metabolized by a mammalian enzyme to a mutagenic substance, unlike most nitro aromatic compounds. Besides, it is not intrinsically mutagenic to the tester strains employed in this study. These findings are toxicologically significant in view of the long duration of antihypertensive therapy.

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