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Diminished toxicity of C-1748, 4-methyl-9-hydroxyethylamino-1-nitroacridine, compared with its demethyl analog, C-857, corresponds to its resistance to metabolism in HepG2 cells☆

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ABSTRACT

The narrow "therapeutic window" of anti-tumour therapy may be the result of drug metabolism leading to the activation or detoxification of antitumour agents. The aim of this work is to examine (i) whether the diminished toxicity of a potent antitumour drug, C-1748, 9-(2′-hydroxyethylamino)-4-methyl-1-nitroacridine, compared with its 4-demethyl analogue, C-857, results from the differences between the metabolic pathways for the two compounds and (ii) the impact of reducing and/or hypoxic conditions on studied metabolism. We investigated the metabolites of C-1748 and C-857 formed in rat and human liver microsomes, with human P450 reductase (POR) and in HepG2 cells under normoxia and hypoxia. The elimination rate of C-1748 from POR knockout mice (HRN) was also evaluated. Three products, 1-amino-9-hydroxyethylaminoacridine, 1-aminoacridinone and a compound with an additional 6-membered ring, were identified for C-1748 and C-857 in all studied metabolic systems. The new metabolite was found in HepG2 cells. We showed that metabolic rate and the reactivity of metabolites of C-1748 were considerably lower than those of C-857, in all investigated metabolic models. Compared with metabolism under normoxia, cellular metabolism under hypoxia led to higher levels of 1-aminoacridine and aza-acridine derivatives of both compounds and of the 6-membered ring metabolite of C-1748. In conclusion, the crucial role of hypoxic conditions and the direct involvement of POR in the metabolism of both compounds were demonstrated. Compared with C-857, the low reactivity of C-1748 and the stability of its metabolites are postulated to contribute significantly to the diminished toxicity of this compound observed in animals.

1. Introduction

The compound 9-(2′-hydroxyethylamino)-4-methyl-1-nitroacridine, C-1748 (Fig. 1), belongs to a group of 1-nitroacridine antitumour agents developed in our department [1]. Strong cytotoxic activity against colon cancer cell lines (HCT8 and HT29) [2] and high antitumour activity against several prostate (LnCaP, JCA, PC3, TSU) and colon carcinoma xenografts (HCT8) in nude mice [3–5] along with low mutagenic potential and slight myelosuppressive properties [6] allowed the selection of C-1748 (Capridine β) for preclinical studies.

Our previous investigations on the mode of action of the 9-amino-1-nitroacridines, particularly the registered antitumour drug Nitracrine® (Ledakrin) [7] (Fig. 1), showed that this drug required metabolic activation before exhibiting any cytotoxic or

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Abbreviations: DTT, dithiothreitol; HLM, human liver microsomes; HRN, Hepatic Reductase Null mouse; POR, NADPH-P450 oxidoreductase; RLM, rat liver microsomes; WT, wild type.

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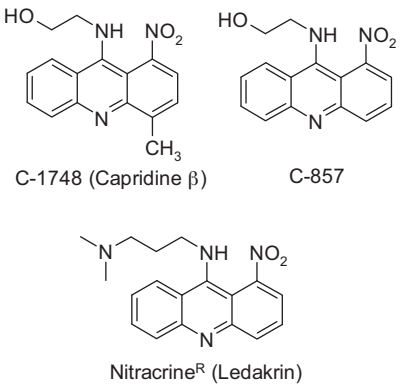

Fig. 1. Chemical structures of anti-tumour 9-amino-1-nitroacridines. Included are the compounds C-1748 and C-857 studied in the present work and the previously studied drug, Nitracrine®.

antitumour activity. Activation resulted in covalent binding of the drug to DNA and other cellular macromolecules [8–10]. The role of metabolism in keeping the balance between antitumour activity and toxicity of 1-nitroacridines is still not well understood. Wilson et al. suggested that the high susceptibility of the 1-nitro group to reduction was the major reason for the antitumour potency of the 9-amino-1-nitroacridines in hypoxic cells [11,12]. However, there was no clear relationship between the electrochemical potential of reduction and the cytotoxicity of the 4-substituted nitroacridines; this finding was in distinct contrast to simpler nitroheterocycles such as nitroimidazoles [13]. Other investigations showed that the values of the reduction potential were close for all isomers of nitroacridine [14], whereas the high susceptibility to metabolism was strongly specific for the 1-nitro isomer only [15,16].

We showed previously that Nitracrine® easily underwent metabolic transformations in the presence of rat liver microsomes and the oxidoreductase enzymes DT-diaphorase and xanthine oxidase [17]. The major products of Nitracrine® metabolism (Fig. 2) were shown to be neither 1-nitrozo, **a**, nor 1-N-hydroxy, **b**, derivatives of 9-aminoacridine. One main metabolite, which was observed in all of the studied metabolic systems, contained a new five-membered heterocyclic ring between positions 1 and 9 of the acridine core, **2**. The metabolite with a 6-membered ring, **3**, was also produced by the microsomes. In addition, the identification of products **4** and **5** obtained with a thiol derivative, dithiothreitol, DTT, indicated that nucleophilic substitutions at positions *ortho* and *para* to the nitro group occurred in the acridine ring before or concurrently with cyclisation.

This specific pathway for the metabolic transformation of Nitracrine® seemed to affect the high cytotoxic and antitumour activity of the new derivatives of 9-amino-1-nitroacridine synthesised in our laboratory. The 9-hydroxyethylamino analogues of Nitracrine®, compounds C-857 and C-1748 (Fig. 1), were characterised by a broader spectrum of antitumour activity. Particularly, C-1748, the 4-methyl derivative of C-857, was active against a wider set of carcinoma xenografts [4,5] and demonstrated outstanding properties in preclinical studies. Preclinical toxicology tests performed in rodents and dogs [18,19] revealed that this compound was significantly less toxic than a set of non-methyl derivatives of 9-amino-1-nitroacridine, particularly those of its direct analogue, C-857.

The present studies were designed considering the results related to the molecular mechanism of Nitracrine® metabolism mentioned above [17] and to the lack of a clear relationship between cytotoxicity and the reduction potential of the 4-substituted Nitracrine® analogues [13]. In contrast to the works mentioned above, we are interested in the role of metabolism in the toxicity of the 9-amino-1-nitroacridines because several representatives of this group expressed extremely high toxicity in animals [20]. We considered the question of whether the diminished toxicity of C-1748 in comparison to the high toxicity of the non-methyl analogue, C-857, might result from differences in the pathways involved in metabolising the two compounds. We also intended to elucidate the role of reducing and hypoxic conditions in the metabolism of both drugs.

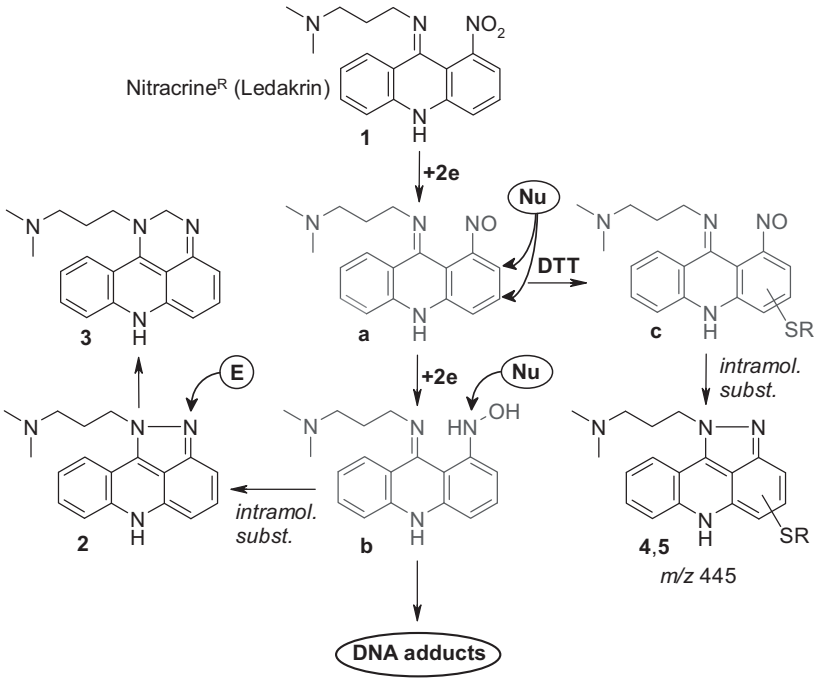

Fig. 2. The pathway proposed for metabolic transformation of Nitracrine® (on the basis of Scheme 1 in Ref. [17]). Reactive intermediates of metabolic reduction, nitrozo (a and c), and hydroxyamino (b), derivatives underwent nucleophilic substitution in the acridine ring and on the activated nitrogen atom. Chemical structures were determined for the final metabolites 2–5.