In vitro effects of Celiptium and MR 14504 on mature rat Leydig cell testosterone production


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Abstract

Percoll-purified mature rat Leydig cells have been used to evaluate the testicular toxicity of two highly potent intercalating agents (Celiptium and MR 14505). Testosterone secretion in the absence and in the presence of human chorionic gonadotropin (hCG) was measured to assess Leydig cell function. Celiptium and MR 14504 induce time- and dose-related inhibitory effects on the production of testosterone by Leydig cells, both in the presence and in the absence of hCG, whatever the concentration of hCG used. We have observed that MR 14504 is about 5 times more potent as an inhibitor of rat Leydig cell steroidogenesis than Celiptium without inducing any cell toxicity. The present study indicates that the Leydig cell is an additional potential site for the primary toxic effects of these drugs in the adult rat testis.

Abbreviations: LH, luteinizing hormone; hCG, human chorionic gonadotropin

Introduction

Numerous relationships between the testicular cell types and a complex hormonal regulatory system with both positive and negative feedback controls are required for the full implementation of mammalian spermatogenesis (Benahmed et al., 1985; Matsumoto, 1989; Boujrad et al., 1992; Carreau, 1994). Radiotherapy, in conjunction with chemotherapy or not, is very effective in treating human cancers, but it is now recognized that some of the antitumoral drugs used (antimitotics, alkylating and intercalating agents, etc.) induce side-effects, especially on the highly sensitive dividing germ cells, and cause temporary or permanent azoospermia (Audoux et al., 1986; Meistrich, 1986; Steinberger and Klinefelter, 1993; Marmar, 1994). Indeed, a variety of chemical substances may affect the testis either directly (testicular functions) or indirectly by altering gonadotropin secretion (Pearce et al., 1986; Levent and Robertson, 1987; Chapin et al., 1990; Kovacevic and Sarac, 1993). It is likely that mammalian Leydig cells will be affected (Johnson et al., 1984; Carreau et al.,
and the consequent impairment of androgen production will block the future recovery of spermatogenesis, for which both FSH and testosterone are required (Steinberger, 1971). Numerous studies have shown that drugs acting on the central nervous system (Phillips et al., 1985; Thomas and Keenan, 1986), alkylating and intercalating agents, and antimitotic drugs (Johnson et al., 1984; Vawda and Davies, 1986; Carreau et al., 1988; Klinefelter et al., 1994; Vawda, 1994), as well as metals (Lasey and Phelps, 1991; Wahba et al., 1994; Zirkin et al., 1985) can under certain conditions inhibit gonadotropin release and therefore produce testicular injury, leading to alteration of reproductive function.

Blood hormonal assays and cell functional tests are mandatory for detection of testicular toxicity, but they are not always sufficient to identify the site of toxic effects and to explain the mechanism of toxicity. Even if no predictive conclusions in humans can be drawn from experimental studies conducted in rodents, it is important to define the side-effects of new drugs, especially on testicular function. Thus the development of models to elucidate the toxic effect of drugs, to complete the conventional hormonal studies, is absolutely required, especially for better understanding of the sequence of morphological, biochemical and molecular changes produced by exposure to chemical injury, not only in terms of damage to the spermatogenetic process (Au roux et al., 1986) but also to testicular somatic cells (Carreau et al., 1988).

In the present study, Percoll-purified adult rat Leydig cells were used to evaluate the in vitro effects of two ellipticine-related drugs: Celiptium (9-hydroxy-2,5,11-trimethyl-6H-pyrido[4,3-b]carbazolium acetate; NSC-264137) and MR 14504 (9-chloro-4-hydroxy-2,3,5,11-tetramethyl-1H,6H-pyrido[3,2b]carbazole methanesulfonate; Robba et al., 1990). These intercalating agents, and especially MR 14504, are very potent both in vitro and in vivo in leukemia and melanoma as well as in mice ovary carcinoma (Lancelot et al., 1986; and personal communication). The rationale of this work was to compare the side-effects of the two drugs on testosterone production by mature rat Leydig cells incubated either with or without luteinizing hormone (LH) and human chorionic gonadotrophin (hCG); in addition, a cytotoxicity test was performed to differentiate between nonspecific (apoptotic cell death) and specific effects (related to the drug action).

Materials and methods

Chemicals

Celiptium (elliptinium acetate) was provided by Pasteur Vaccins (Paris) and MR 14504 was purchased from CERMN (France). Since the discovery of the antitumor properties of ellipticine, numerous studies have been done on the related biological functions of pyrido[3,2-b]carbazoles (Figure 1). Celiptium is widely used in cancer therapy; however, the compound is highly toxic. Consequently, other pyrido[3,2-b]carbazole derivatives which present the same structural characteristics (i.e., intercalating agents) have been prepared, among them MR 14504, which is very potent both in vitro and in vivo. Powdered Ham F12 and DME were purchased from Seromed (France); collagenase–dispase was from Boehringer (Mannheim, Germany). Soybean trypsin inhibitor (STI) and deoxyribonuclease I were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and highly purified hCG from Organon (France). Testosterone antibody was purchased from bioMerieux (France), and [1,2,6,7-3H]testosterone from Amersham (UK). Solvents and chemicals were of analytical grade from Merck (Darmstadt, Germany). All other reagents used for cell purification and culture were as described elsewhere (Papadopoulos et al., 1985).