A Sequential Analysis of Meiosis in the Male Mouse Using a Restricted Spermatocyte Population Obtained by a Hydroxyurea/Triaziquone Treatment

J.L. Oud, J.H. de Jong, and D.G. de Rooij

1 Institute of Genetics, Department of Cytogenetics and Population Genetics, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, the Netherlands, and
2 Department of Histology and Cell Biology, State University Medical School, Utrecht, the Netherlands

Abstract. A method is described to restrict the spermatocyte population in mice and other rodents using hydroxyurea (HU) and triaziquone (T). HU affects cells in S-phase, whereas T is an agent especially active on spermatogonia and not on spermatocytes. An application of three i.p. HU injections with 12 h intervals, followed about nine days later by one i.p. T injection creates two large gaps in the spermatogenic line. The two gaps enclose a small, well-defined group of primary spermatocytes in meiotic interphase. - The development of the restricted spermatocyte population is followed day by day. The analysis of meiosis in male mice has revealed the correct sequence of meiotic, and especially prophase I stages. On account of clearly visible differences in chromosome morphology the diplotene stage could be divided into three periods. It is suggested to use the following nomenclature: pre-diffuse diplotene, diffuse diplotene and post-diffuse diplotene. The experiment was also informative about the timing of the stages in spermatocyte development by correlating the days at which the successive stages were observed with the corresponding stage of the epithelial cycle. The calculation of the position and duration of the diffuse diplotene, enables us to put forward a proposal about the significance of the diffuse diplotene. - A combination of the HU/T method with cell separation techniques provides good perspectives for detailed biochemical studies on processes taking place during meiosis.

Introduction

Although there exists a vast literature about meiosis, many processes which take place especially during prophase I are not fully understood. Many problems can only be solved by combining the results of microscopical analyses and biochemical techniques. For such studies the availability of restricted and well-defined populations of spermatocytes is a prerequisite. Recently a few methods have been described to obtain germ cell populations of known developmental
age. All methods but one make use of sedimentation velocity techniques, which yield groups of cells with a rather great difference in age between the least and most advanced spermatocytes. The remaining method is based on transillumination of freshly isolated, unstained seminiferous tubules (Parvinen and Vanha-Perttula, 1972). These authors observed that differences in light absorption provide the possibility to identify the stages of the cycle of the rat seminiferous epithelium. The transillumination technique is laborious and not suitable to use on mouse tubules as these are much thinner than those of the rat.

The present paper describes a method which restricts the spermatocyte population using hydroxyurea (HU) and triaziquone (T). Both compounds are able to kill spermatogonia. The final result of successive treatment with HU and T is a small, definite group of surviving primary spermatocytes in interphase. The development of the restricted spermatocyte population is followed day by day. This approach makes it possible to analyse the correct sequence of meiotic prophase stages, and gives detailed information about the timing of the stages.

Material and Methods

1. Procedure of the Hydroxyurea/Triaziquone (HU/T) Treatment. The animals used were young adult Swiss random-bred mice (CPB/TNO, Zeist, the Netherlands). 40 Mice received 3 i.p. doses of 350 mg hydroxyurea (BDH Chemicals Ltd) per kg bodyweight freshly soluted in physiological saline. Nine days and 4 h after the first HU injection they received 1 i.p. dose of 0.25 mg triaziquone (2, 3, 5-tris(aziridinyl)-p-benzoquinone; Trenimon®, Bayer) per kg bodyweight soluted in physiological saline. Both compounds were diluted so that a mouse weighing 25 g received 0.1 ml of the solution. The mice were killed 1 to 13 days after T injection (three mice each day). One and a half testes were used for chromosome preparations and the remaining half a testis for histological examination.

2. Preparation for the Histological Study. The material to be studied histologically was fixed in Bouin's fixative; 5 µm paraffine sections were made, and stained with periodic acid-Schiff-hematoxylin.

3. Preparation for the Cytological Study. After removing the tunica albuginea, the seminiferous tubules underwent a hypotonic pretreatment with 1% sodium citrate during 30 min at room temperature. Fixation in a mixture of absolute ethanol and glacial acetic acid (3:1, v/v) (temperature of the mixture: 4°C) lasted 10 min at room temperature and after refreshing the fixative another 30 min at 4°C. Next, a cell suspension was made by dissolving the tubules in 30% acetic acid. After centrifugation (10 min at 200 x g) the pellet was resuspended in the fixative. The suspension was dropped onto ice-cooled, grease free, wet slides and spread on a hot plate. The slides were stained during 2 h in 2% lacto propionic orcein, and examined with phase contrast optics.

Results

1. Hydroxyurea/Triaziquone Treatment

According to the model of Huckins (1971), Oakberg (1971) and de Rooij (1973) spermatogonial multiplication begins with divisions of the so called. A_{ns}, A_{pr} and A_{al} spermatogonia. Once every cycle of the seminiferous epithelium, A_{1} spermatogonia are formed by A_{al} cells. The A_{1} spermatogonia arise in stages