INFLUENCE OF TRIAMCINOLONE AND SOMATOSTATIN ON MORPHOMETRIC PARAMETERS OF CULTURED INTESTINAL MUCOSA

A. SCHNEIDER, G. GRÖSCH, E. F. STANGE, AND H. DITSCHUNEIT

Department of Internal Medicine, University of Ulm, Ulm, Germany

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SUMMARY

Mucosal biopsies from rabbit ileum were organ cultured for 24 h. The influence of triamcinolone and somatostatin on villus height and diameter as well as crypt depth and the number of mitoses was measured at various times during 24 h of culture as indices of cell proliferation and tissue maintenance. It could be shown that triamcinolone reduced cell proliferation slightly but preserved mucosal structure in organ culture. Somatostatin inhibited crypt cell proliferation, without any effect on other morphological parameters.

Key words: organ culture; rabbit; triamcinolone; somatostatin; morphometry; mitoses; intestine.

INTRODUCTION

Proliferation and differentiation along the crypt-villus axis are regulated by various factors including villus size, nutritional and hormonal status, and innervation (5,6,13,17,21,34). Among other hormones, the role of glucocorticoids is of paramount importance in intestinal cell development. In the embryonic and fetal intestine these steroids induced rapid brush border maturation (4,35) whereas in the adult intestine both an enhanced absorptive capacity for Na+ (33) and galactose (32) and an increase in brush border enzyme activity (1,37) have been demonstrated. Despite these dramatic effects there was no change in villus height (1) or villus transit time (32). In addition, the growth response seems to vary with respect to the type of glucocorticoid used and the in vitro or in vivo system under study (1,12,32,41).

The importance of somatostatin is more uncertain, because the physiological effect of this peptide on gut mucosa is unclear. In pharmacological doses somatostatin was documented to inhibit the release of gut peptides, gastrointestinal secretions, and absorption (19). In addition, the hormone diminished intestinal DNA synthesis and cell division in the living rat (18), particularly after partial resection (11,28), and suppressed growth and differentiation in cultured rabbit mucosa (39).

Most experiments, however, were based on hormone administration in vivo or elimination of the hormone-producing organ. To exclude interfering effects by the given hormone through changes in the endocrine state or absorptive condition of the living animal, mucosal organ culture (42) was applied to directly study proliferation and tissue maintenance. Both were measured by a selection of morphometric and cell kinetic parameters which were recently adapted to the organ culture technique (40).

MATERIALS AND METHODS

New Zealand White rabbits aged 2 to 3 mo., weighing 1.0 to 1.5 kg were anesthetized with pentobarbital. Thirty centimeters of the terminal ileum was excised. The gut was rinsed with ice-cold saline and biopsies were obtained by cutting 1 μ 2-mm pieces of mucosa with a scalpel. The biopsies were immediately placed on grids floating on 1 ml of medium in Falcon organ culture dishes and cultured for 24 h according to the method of Trier (42), with modifications as previously described (37). The culture medium consisted of 90% Trowell's T8, 10% fetal bovine serum, penicillin (100 U/ml), and Gentamicin (100 μg/ml). The atmosphere consisted of 95% oxygen:5% carbon dioxide. Mucosal viability for 24 h was shown previously by brush border enzyme stability (39), an increase in 3-hydroxy-3-methylglutaryl Coenzyme A reductase activity (38), and a linear [%H]thymidine and [%H]leucine incorporation in DNA and protein, respectively (38). Fresh biopsies and cultured mucosa were processed according to Clarke (7) for morphometry, and stained with Feulgen reagent for counting the metaphases as detailed in a recent report (40).

The metaphase arrest was achieved at an optimal concentration of 1 μg/ml vinblastine to the medium 2 h before the end of the respective culture period (40). The microdissection of crypt and villi was performed under a binocular microscope (Zeiss) according to Lorenz-Meyer et al. (22,23).

Subsequently, height and diameter of villi, crypt depth and number of villi per square millimeter were measured, and the metaphases per crypt were counted. An average
value of the morphometrical data was derived from 10 villi and crypts randomly dissected from a total of 10 biopsies per treatment group. In one series of cultures all five treatment groups were compared, i.e. 50 biopsies were obtained for culture from each rabbit. The means ± SEM were calculated from the individual average values of six rabbits in each group. The statistical evaluation was based on Student's t test. For multiple comparisons corrections were made according to Holm (10).

Cyclic somatostatin, kindly supplied by Serono, Freiburg, FRG, was dissolved in ethanol and added to the medium at the appropriate concentrations. Water-soluble triamcinolone, a well absorbable glucocorticoid hormone essentially devoid of mineral corticoid activity, was obtained from Sigma, München, FRG. Rabbits were supplied by Fa. Thomae, Biberach, FRG. Organ tissue culture dishes were from Falcon, Cockeysville, MD, and culture medium from Gibco Biocult, Karlsruhe, FRG. All other materials were obtained from Fa. Merck, Darmstadt, FRG.

**RESULTS**

In the control group, the villus diameter was reduced by 34% after a 24-h culture period (P < 0.05, Fig. 1 A), and the height of the villi was diminished by 54% (P < 0.025, Fig. 1 B). The diameter of the villi was not changed by addition of triamcinolone (Fig. 1 A). Nevertheless, the addition of triamcinolone in concentrations of 10⁻⁷ and 10⁻⁵ M exhibited a striking protective effect on cultured ileal mucosa. The height of the villi was reduced significantly less than in the control group after 24 h of culture (Fig. 1 B). This effect of triamcinolone was observed from 6 up to 24 h of culture and at both concentrations. At 24 h the villus height was 0.278 mm at 10⁻⁷ M (P < 0.001 compared to controls) and 0.293 mm at 10⁻⁵ triamcinolone (P < 0.002 compared to controls), whereas the control group was reduced to 0.185 mm (P < 0.025 compared to 0 h). Nevertheless, compared to in vivo dimensions the original height could not quite be preserved by both concentrations of triamcinolone (P < 0.005).

In addition to the height of villi, the depth of the crypt decreased by 30% in the control group (P < 0.05, Fig. 2 A). With 10⁻⁷ M triamcinolone the depth of the crypt increased from 0.086 to 0.112 mm (P < 0.01) and with 10⁻⁵ M up to 0.120 mm (P < 0.002) after 24 h of culture, which is identical with the crypt depth in fresh biopsies (Fig. 2 A). However, the number of villi per square millimeter of the control group did not change during culture in control medium, nor was it altered by the addition of triamcinolone (Fig. 2 B).

The metaphase arrest in vivo 2 h after an intravenous injection of 1 mg/kg vincristine in a group of six rabbits resulted in 15.6 ± 0.2 metaphases per crypt. In comparison, spontaneous arrests without vincristine were 2.8 ± 0.1 (P < 0.001). In contrast, only 0.16 ± 0.04 spontaneous metaphases could be counted after 2 h of culture. The addition of 1 µg/ml vincristine

**FIG. 1.** Influence of triamcinolone on diameter (A) and height of villi (B) as a function of time of culture. Results are expressed as means ± SEM. Symbols for control and treatment groups are given in the lower panel. Significant levels are calculated after 24 h of culture (see Results).

**FIG. 2.** Influence of triamcinolone on depth of crypt (A) and number of villi/mm² (B) as a function time of culture. Results are expressed as means ± SEM. Symbols for control and treatment groups are given in the lower panel. Significant levels are calculated after 24 h of culture (see Results).