Light Microscopic Examination of the Jejunum after Long-Standing Azathioprine Treatment

Azathioprine (Imuran) is known to inhibit RNA and DNA synthesis in a complex manner[1]. It also belongs to the cytotoxic drugs[2]. Although nausea and malaise are well-known side-effects, only very few investigations of the gastrointestinal tract have been done in humans[3,4]. We have studied the effects of long-standing azathioprine treatment on the jejunal mucosa. The enterocyte is known for its very high cell turnover and changes in the mitotic activity of the crypt-cells can secondarily affect the morphology and the size of the villi.

Jejunal biopsies have been carried out with the multipurpose Rubin biopsy tube at the angle of Treitz in 2 females and 5 males, aged from 18 to 44 years, after an overnight-fast. All of them had cadaver kidney transplants in the past. Only very reliable and well controlled patients were included in this study. To eliminate influence of uremia on the gastrointestinal tract, we chose patients whose state of transplant was stable at the time of biopsy (creatinine clearance between 35 and 90 ml/min, average 59.3) and whose transplant has been done more than 1 year ago, namely 58–169 weeks before. The patients were treated permanently with azathioprine (average dose in last 6 months before biopsy 50–150 mg per day, total dose varying from 45,370 to 95,125 g) and steroids (7.5–30 mg prednisone a day, total dose 10,025 to 34.66 g). The biopsy specimens were oriented on a filament mesh, immediately fixed in Bouin's solution for 1 to 3 h and then kept in 70% alcohol. After fixation they were embedded in paraffin wax. Serial sections were done parallel to the cut edge so that full thickness mucosal sections were obtained with vertically-oriented villi throughout. The sections were stained with Haematoxylin Eosin, Alcian Blue, Argentafin and PAS in the Department of Pathology, where the sections were also examined by light microscopy. In 3 patients oculomicrometric measurements of the villi were done. The height of the villi was measured from the junction of the crypt and the villus to the tip of the villus.

Surprisingly enough, the small bowel mucosa, by light microscopy, showed normal slender villi with normal cell differentiation and normal villus gland ratio. In the crypts the glandular elements and the mitotic activity appeared normal. There was no edema or cellular infiltration of the lamina propria. The results of the oculomicrometry are shown in the Table. 3 measurements have been done for each patient and these were all within normal range (320–570 μm, SHINER and DONIACH[5]).

The fact that no changes could be demonstrated by light microscopy and oculomicrometry raises a number of questions. It is possible that our methods were not sensitive enough. Electronmicroscopy, histochemistry and enzyme studies will be done in the near future. It could also be that the drug provokes early changes with diminution of the mitotic counts in the crypts as has been described with methotrexate[6], but that after a while the epithelium adapts to the drug and gradually shows normalization of the mitotic activity. Also, the azathioprine metabolite thioinosinic acid, which is responsible for the cytotoxic effect[1], could be accumulated in other tissues than the gastrointestinal tract. Unfortunately, to date no method allows one to measure these metabolites in small tissue pieces. Finally, it may well be that in patients who are more sensitive to the drug and show signs of side-effects, the small bowel mucosa does not look normal. Our patients, however, tolerated the drug very well and did not show any side-effects. No signs of bone-marrow depression could be detected. The leucocyte count was between 6000 and 13,000/mm (average 8000) and the hematocrit between 35 and 50% (40,9). It is fortunate that immunosuppression with azathioprine occurs at doses below those which have toxic effects on bone-marrow and intestinal mucosa. We do not know how much the corticosteroids could account for the negative results, because the influence of steroids on the turn-over of the jejunal epithelial cells has not been studied.

Results of the measurements of villus height

2	Villus height	337 μm	389 μm	399 μm
6	Villus height	370 μm	405 μm	459 μm
7	Villus height	389 μm	414 μm	430 μm

Zusammenfassung. Bei 7 Nierentransplantierten, die länger als 1 Jahr mit mittleren Dosen Imurel und Prednison behandelt wurden, ergaben lichtmikroskopische Untersuchungen von Dünndarmbiopsien keine Anhaltspunkte für Epithelschädigung.

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Specificity of Antibodies to Arginine-Vasopressin Raised with Succinylated Poly-L-Lysine as Carrier

Antibodies to neurohypophysial hormones were first obtained by injecting rabbits either with unmodified oxytocin[1] or vasopressin[2], or with conjugates obtained by coupling covalently oxytocin (OCT) or lysine-vasopressin (LVP) to serum albumin[3–6].

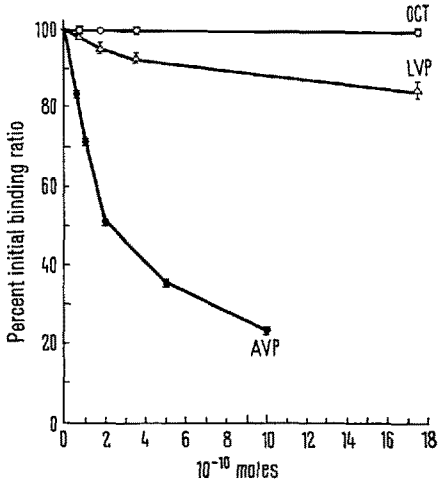
Branch-chain copolymers of succinylated poly-L-lysine and short peptides such as angiotensin I and II[7–10] or even smaller compounds such as triiodothyronine[11] have been successfully used to raise antibodies dotted with both a high affinity and a great specificity. The use of

a homopolymer of amino acid is of advantage since it is in itself non-immunogenic and thanks to the repetition of the same residue does not introduce an element of heterogeneity at the point of attachment of the peptide. The antibodies produced are consequently essentially directed against the haptenic side-chains.

We report here the results obtained with a copolymer of succinylated poly-L-lysine and arginine-vasopressin (AVP)[12] prepared by a modification of the previously described method[13]. 6 mg succinylated poly-L-lysine was dissolved in 1 ml H_2O, then 20 mg AVP, 1 ml Dioxane and 5 mg Triethylamine were added. After adjustement of the pH to 5.5 with methanesulfonic acid, a clear solution was obtained to which 12 mg 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride was added. This mixture and a similar one in which AVP was omitted were stirred in the dark at room temperature for 24 h; at that time a fine white precipitate appeared only in the mixture with AVP. The suspension was dialyzed for 48 h against water and then lyophilized. 8 mg of the copolymer was suspended and finely dispersed in 2.5 ml NaCl 0.9% and emulsified in an equal volume of complete Freund's adjuvant. Rabbits were immunized with 1 ml of this emulsion distributed into toe-pads, subcutaneously and intramuscularly.

After 4 months, antibodies to AVP could be detected in 3 out of 4 rabbits. A radioimmunoassay technique was then developed. AVP was labelled with iodine-125 according to the method of HUNTER and GREENWOOD[14] after adjusting the correct quantities of the reagents. AVP labelled at a low specific activity, in order to obtain essentially only monoiodotyrosine derivatives formation, was purified and its specific activity greatly increased by high-voltage electrophoresis[15].

Equilibrium between various quantities of AVP and a fixed amount of diluted antiserum in presence of trace amount of labelled AVP was already reached after 4 h at 4 °C in 1 ml *Tris*-acetate buffer (0.1 *M*, pH 7.5) with 1 mg Lysozyme per ml. Separation of antibody-bound and free hormone was obtained by adsorption of the latter on Dextran-coated charcoal after addition of 1 ml of a suspension containing 2 g/100 ml charcoal. After a quick mixing the suspension was centrifuged within 5 min for 20 min at 4 °C. The radioactivity of both fractions, supernatant or bound fraction (B) and sedimented charcoal or free fraction (F) were counted and the ratio B/F and B/B + F computed. Similar incubations were made with increasing amounts of lysine-vasopressin (LVP) or oxytocin (OCT) instead of AVP. Respectively 35 times more LVP was required to achieve a similar displacement of the tracer, while in the range of concentration tested OCT did not compete with AVP for the binding sites of the antibody (Figure). OCT differs from AVP in position 3 and 8 where respectively phenylalanine[3] and arginine[8] are replaced by isoleucine[3] and leucine[8]. It is thus not surprising that the antigenicity of these peptides is quite different, as it has been observed by other workers[4,5]. On the other hand, LVP differs from AVP only by the substitution of arginine by lysine in position 8, that is to say the replacement of a guanidino group by a methyl-amino group. The antibodies raised against AVP, using poly-L-lysine as carrier, show this difference and have a stronger affinity for AVP than for LVP, as do physiological receptors, since AVP is more potent than LVP on a per mole basis in all biological assays[16,17].



Comparison of the displacement of arginine-vasopressin-I^{125} bound to antibody by arginine-vasopressin (AVP), lysine-vasopressin (LVP) and oxytocin (OCT).

Résumé. Des anticorps dirigés contre l'arginine-vasopressine ont été obtenus chez le lapin après immunisation avec un copolymère ramifié de poly-L-lysine succinylée et d'arginine-vasopressine. La spécificité de ces anticorps est remarquable puisque non seulement l'on n'observe pratiquement pas de réaction croisée avec l'oxytocine, mais en plus leur affinité pour la lysine-vasopressine est environ 35 fois plus faible que pour l'arginine-vasopressine.

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