INFLUENCE OF HEMITHYROIDECTOMY ON BROMODEOXYURIDINE INCORPORATION INTO DNA OF RAT THYROID FOLLICULAR CELLS

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Objective. To examine the bromodeoxyuridine (BrdU) incorporation into DNA of thyroid follicular cells (TFC) in the remaining thyroid lobe after hemithyroidectomy (hemiTx) in 1, 2, 3 and 4 weeks time after surgery.

Methods. The experiment was performed on male Wistar rats. The Cell Proliferation Kit (Amersham, UK) was used in order to detect the incorporated BrdU. The BrdU incorporation was expressed as a BrdU labelling index ($BrdU_{LI}$; a number of BrdU-immunopositive TFC per 1000 TFC).

Results. 1. No statistically significant changes of $BrdU_{LI}$ were observed between the particular groups of sham-operated (shamTx)-rats in 1, 2, 3 and 4 weeks time after surgery, and in comparison of each of them to the controls (at time "0"); 2. In the first 2-week period after hemiTx, an increasing effect of that surgical procedure on $BrdU_{LI}$ value was observed (the highest $BrdU_{LI}$ value was detected 2 weeks after hemiTx); 3. In the third and fourth week after hemiTx, a decrease of $BrdU_{LI}$ value was observed, as compared to $BrdU_{LI}$ groups (in 1- and 2-week time after hemiTx), and to the controls (at time "0"); 4. An increase of weight of contralateral lobe was shown in 1, 2, 3 and 4 weeks after hemiTx in comparison to thyroid lobe weight in intact rats.

Conclusions. During the first 2 weeks after hemiTx, the thyroid growth in the remaining thyroid lobe seems to ensue by hyperplasia mechanisms. The thyroid growth processes during subsequent 2 weeks (3rd and 4th) could result from other mechanisms – for example, from hypertrophy.

Key words: Bromodeoxyuridine – Labelling index – Hemithyroidectomy – Rat thyroid – Growth processes

5-Bromo-2'-deoxyuridine (BrdU) is a thymidine analogue which is incorporated into DNA in the S phase of cell cycle (synthesis of DNA) (YU et al. 1992). 5-Bromo-2'-deoxyuridine incorporation can be quantified by fluorescent or chromophoric quenching of dyes, bound to DNA, or with antibodies to BrdU. The turning-point in the studies, using BrdU technique, was the introduction of antibodies to BrdU (GRATZNER et al. 1975; 1976; 1978) including monoclonal ones (GRATZNER 1982) which offered a new immunochemical method of BrdU detection. It improved the accuracy of the method. In the last decade the development of methods for detecting incorporated BrdU by electron microscopy has been described (THIRY 1988; 1992).

It is worth to emphasize that BrdU enhances the sensitivity of cells to X-rays (DJORDJEVIC and SZYBALSKI 1960; YU et al. 1992). This substance is used in radiotherapy regimes in the treatment of human malignant tumours (YU et al. 1992). Recently, it was found that BrdU increased the cytotoxicity induced by 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin in human glioma cells (MANCINI et al. 1999). Furthermore, it has been shown that BrdU is a substance

which is characterized by a great genotoxicity. It has been demonstrated that BrdU causes the induction of sister-chromatid exchanges, mutations, an inhibition of cell proliferation and an expression of fragile sites in the human genome (Morris 1991).

Moreover, it is worth recalling that the stimulatory effect of hemithyroidectomy (hemiTx) on growth processes in the remaining thyroid lobe is a well-known observation (Logothetopoulos and doniach 1955). Therefore, the surgical procedure of hemiTx can be a good way to assess morphological, cytological and histological changes which accompany the proliferative processes.

The aim of the study was to examine BrdU incorporation into DNA of thyroid follicular cells (TFC) in the remaining thyroid lobe after hemiTx. The study has allowed to assess the dynamics of changes occurred in the gland in consequence of hemiTx.

Materials and Methods

The experiment was performed on one hundred and twenty (120) male Wistar rats, weighing 95±10 g each. The rats were divided into thirteen (13) groups, the Groups I to X consisting of nine (9) animals and the Groups XI – XIII consisting of ten (10) animals. The following time points were considered: time "0", 1, 2, 3 and 4 weeks after hemiTx or shamTx. BrdU incorporation was expressed as a BrdU labelling index ($BrdU_{LI}$; the number of BrdU-immunopositive TFC per 1000 TFC). The following groups were considered: **Group I:** [Controls (control group) – right lobe (RL) or left lobe (LL) – time "0"]; **Group II**: [sham-operation (shamTx) – RL or LL – 1 week after the surgery]; **Group III**: [shamTx – RL or LL – 2 weeks after the surgery]; **Group IV**: [shamTx – RL or LL – 3 weeks after the surgery]; **Group V**: [shamTx – RL or LL – 4 weeks after the surgery]; **Group VI** [RL after left-side hemithyroidectomy (L-hemiTx) – 1 week after the surgery]; **Group VII** [LL after right-side hemithyroidectomy (R-hemiTx) – 1 week after the surgery]; **Group VIII** [RL after L-hemiTx – 2 weeks after the surgery]; **Group IX** [LL after R-hemiTx – 2 weeks after the surgery]; **Group X** [RL after L-hemiTx – 3 weeks after the surgery]; **Group XI** [LL after R-hemiTx – 3 weeks after the surgery]; **Group XII** [RL after L-hemiTx – 4 weeks after the surgery]; **Group XIII** [LL after R-hemiTx – 4 weeks after the surgery].

One hour before decapitation, the animals had received an intraperitoneal injection of BrdU (Sigma, USA) in a dose of 50 mg/kg BW. The thyroid lobes were collected and fixed in 4% formalin for 6 hours and, later on, in 70 % ethanol. Then, the procedure of embedding of the thyroids in paraffin wax (using 95 % ethanol, aniline, carboxylene, xylene and finally paraffin) was performed. Next, the paraffin sections (4 μm thick) were immunostained, using the Cell Proliferation Kit (Amersham, UK) in order to detect the incorporated BrdU.

We used the following components of the Cell Proliferation Kit: the nuclease for DNA denaturation which allows antibodies access to BrdU; anti-5-bromo-2'-deoxyuridine monoclonal antibody of murine origin; subclass IgG2a; peroxidase conjugated antibody to mouse immunoglobulin IgG2a (of caprine origin), substrate/intensifier and DAB (3,3'-diaminobenzidine tetrahydrochloride). The substrate/intensifier is an aqueous solution of hydrogen peroxide required for the peroxidase catalyzed polymerization of DAB, together with cobalt chloride and nickel chloride to give blue-black staining at the sites of BrdU incorporation and to intensify and increase the contrast of the resulting stain. Thanks to that procedure, the detection of bound antibody is much achieved.

The $BrdU_{LI}$ values were evaluated in microscopic preparations (counting the number of BrdU-immunopositive TFC in each specimen and expressing of counted BrdU-immunopositive TFC per 1000 TFC). To ensure confidence in the interpretation of experimental results, two sets of controls were always included in each staining procedure: cells not exposed to BrdU (thyroid lobes derived from the rats which did not obtain the intraperitoneal injection of BrdU) and cells stained without anti-BrdU (removal of antibodies to BrdU).

The data were statistically evaluated by the one-way analysis of variance (ANOVA). The statistical significance of differences among the individual groups was evaluated by using the Neuman-Keuls' test (Hinkle et al. 1979). The results, obtained for the right and left lobes in each experimental group, have been jointly shown in Figures and expressed as means ± SEM.

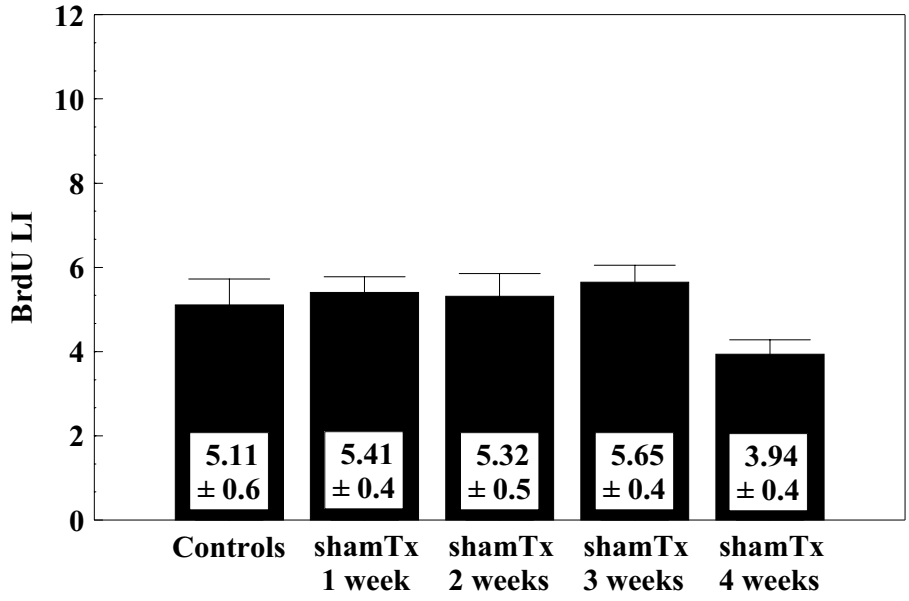

Figure 1
$BrdU_{LI}$ value in thyroid lobes of sham-operated rats. Each value represents the mean ± SEM. The BrdU labelling index ($BrdU_{LI}$) is expressed as the number of BrdU-immunopositive thyroid follicular cells (TFC) per 1000 TFC. shamTx 1 week – after 1 week after shamTx, shamTx 2 weeks – after 2 weeks after shamTx, shamTx 3 weeks – after 3 weeks after shamTx, shamTx 4 weeks – after 4 weeks after shamTx.

Results

No statistically significant changes of $BrdU_{LI}$ were observed between the particular groups of shamTx-rats, after 1, 2, 3 and 4 weeks after the surgery and in comparison of each of them to the control group (Controls) (at time "0") (Figure 1). In the first two weeks after hemiTx, an increasing effect of that surgery on $BrdU_{LI}$ value was observed (the highest $BrdU_{LI}$ value was detected 2 weeks after hemiTx – $p<0.001$ vs. $BrdU_{LI}$ in rats from the control group, $p<0.08$ vs. $BrdU_{LI}$ in hemiTx-rats in 1 week after the surgery; $BrdU_{LI}$ in 1 week after hemiTx – $p<0.02$ vs. $BrdU_{LI}$ in rats from the control group) (Figure 2). In the third and fourth week after hemiTx, we observed a decrease of $BrdU_{LI}$ value, in comparison to $BrdU_{LI}$ in hemiTx-rats in 1 week after the surgery (after 3 weeks and 4 weeks – $P<0.001$ vs. $BrdU_{LI}$ assessed in 1 week after the hemiTx) and also in 2 weeks after hemiTx (after 3 weeks and 4 weeks – $P<0.001$ vs. $BrdU_{LI}$ in hemiTx-rats in 2 weeks after the surgery) (Figure 2). A decrease of $BrdU_{LI}$ value in the third and fourth week after hemiTx was also demonstrated, in comparison to $BrdU_{LI}$ value in animals from the control group (after 3 weeks – $P<0.02$ vs. $BrdU_{LI}$ in rats from the control group, after 4 weeks – $P<0.003$ vs. $BrdU_{LI}$ in animals of the control group) (Figure 2). The lowest $BrdU_{LI}$ value was observed 4 weeks after hemiTx (Figure 2).

In each experimental group, a weight of contralateral thyroid lobe (right or left) after hemiTx was evaluated. A gradual increase of weight of the lobe remaining after the surgery was observed in all time points (1, 2, 3 and 4 weeks after hemiTx) in comparison to the weight of thyroid lobes in intact rats (controls – time "0") [all groups after hemiTx – $p<0.001$ vs. controls] (Figure 3). The highest weight was shown 4 weeks after that surgery (Figure 3).

Discussion

In the present study, BrdU (50 mg/kg BW) was intraperitoneally administered 1 hour before decapitation. Such a procedure is similar to that employed

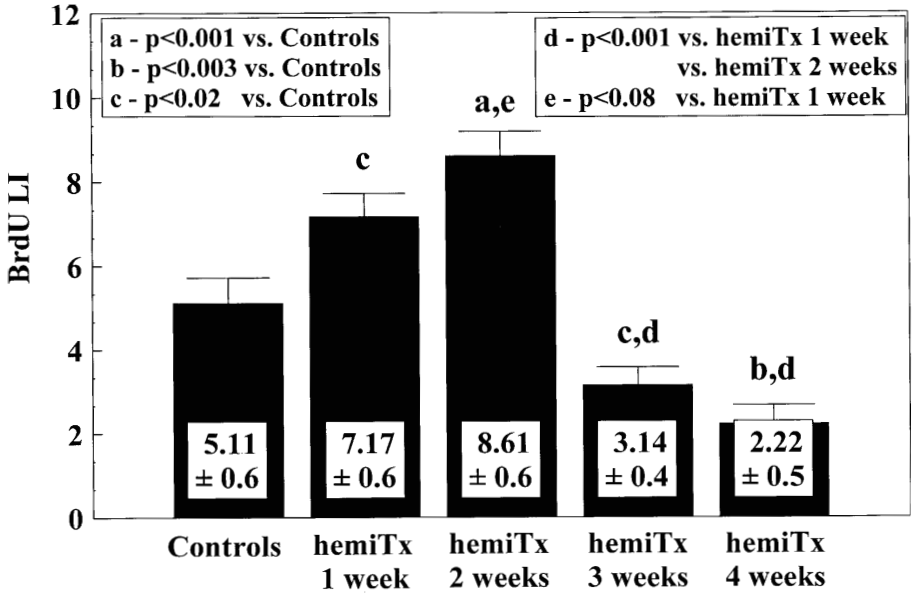

Figure 2

$BrdU_{LI}$ value in the remaining thyroid lobe of hemithyroidectomized rats. Each value is the mean ± SEM. The BrdU labelling index ($BrdU_{LI}$) is expressed as the number of BrdU-immunopositive thyroid follicular cells (TFC) per 1000 TFC. hemiTx 1 week – after 1 week after hemiTx, hemiTx 2 weeks – after 2 weeks after hemiTx, hemiTx 3 weeks – after 3 weeks after hemiTx, hemiTx 4 weeks – after 4 weeks after hemiTx.

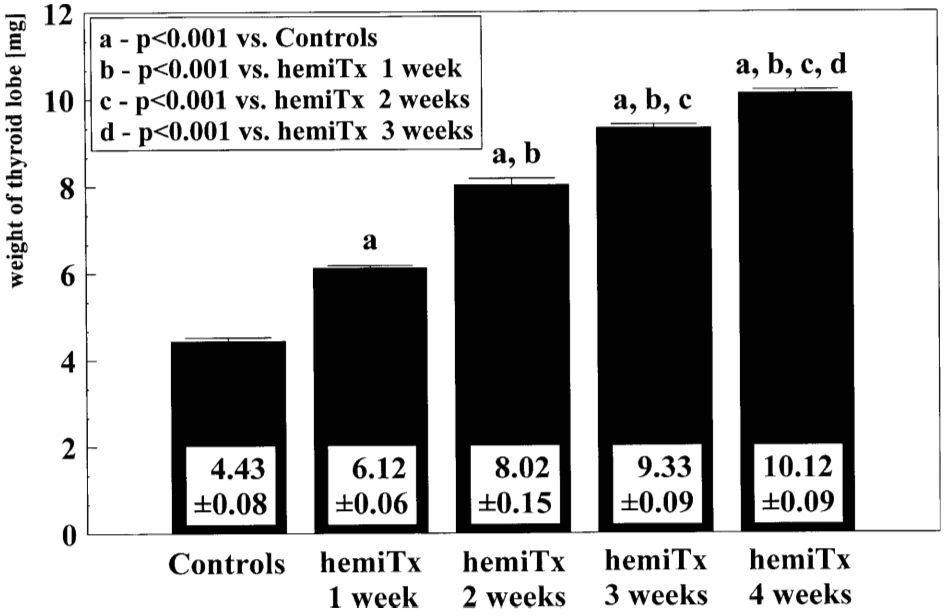

Figure 3

Weight of thyroid lobe in intact rats and of contralateral lobe in rats after hemiTx. Each value illustrates the mean ± SEM. hemiTx 1 week – after 1 week after hemiTx, hemiTx 2 weeks – after 2 weeks after hemiTx, hemiTx 3 weeks – after 3 weeks after hemiTx, hemiTx 4 weeks – after 4 weeks after hemiTx.

in earlier experiments, performed for the purpose of assessment of BrdU incorporation, e.g., into anterior pituitary cells (Pawlikowski and Slowinska-Klencka 1994) or into gastric mucosal cells (Fligiel et al. 1994).

No statistically significant changes of $BrdU_{LI}$ were observed between the particular groups of rats, 1, 2, 3 and 4 weeks after shamTx, and in comparison of each of them to the controls. In the first 2 weeks after hemiTx, we observed an increasing effect of that surgery on $BrdU_{LI}$ value (the highest $BrdU_{LI}$ value was shown 2 weeks after hemiTx). In the second 2-week period (the third and fourth week after hemiTx), a decrease of $BrdU_{LI}$ value was observed (the lowest $BrdU_{LI}$ value was shown in 4 weeks time after hemiTx).

The obtained results indicate a different course of the examined growth processes. It can be assumed that, during the first 2 weeks after hemiTx, the growth processes in the remaining lobe seem to ensue by hyperplasia mechanisms (it is known that BrdU incorporation into DNA occurs in DNA synthesis phase of the cell cycle). In the third and fourth week after hemiTx, a decrease of $BrdU_{LI}$ value was observed. We have concluded that the thyroid growth processes during subsequent two weeks after hemiTx, could result from other mechanisms (different from hyperplasia) – for example, from hypertrophy. In other experiments performed on rats, it was demonstrated that the removal of one thyroid lobe was a cause of compensatory growth processes in the contralateral lobe, ensued by hyperplasia (Lewinski 1981), or by hypertrophy mechanisms (Clark et al. 1976). Moreover, a rapid switch from myocyte hyperplasia to hypertrophy has been shown, which occurs in rat hearts 3-4 days after birth (Li et al. 1996). It is worth stressing that the $BrdU_{LI}$ values – obtained in our experiment – were lower as compared to the index values obtained in some earlier studies (Yoshida et al. 1992). The cited Japanese authors examined growth processes in various human thyroid tumors – e.g., in papillary, follicular and medullary carcinomas, as well as in adenomas, using the *in vitro* BrdU-labelling technique and immunohistochemical staining with anti-BrdU monoclonal antibody (Yoshida et al. 1992). The highest $BrdU_{LI}$ value was observed in the case of papillary carcinoma – 4.6% (Yoshida et al. 1992). In other experiments with use of BrdU i.v. administration, it was demonstrated that $BrdU_{LI}$ values of the canine oral malignant melanoma and fibrosarcoma were 7.5 % and 10.3 %, respectively (Yoshida et al. 1999). The above-mentioned results were approximately 10 times higher than the biggest $BrdU_{LI}$ value, obtained in our present study – in 2 weeks time after hemiTx (8.61 ± 0.6 ‰). However, one should not forget the differences in experimental protocol between these two studies (our present work has been performed on rats *in vivo*).

On the basis of the above cited and our own results, we conclude that the examination with use of the BrdU-labelling technique, may be useful tool for the assessment of proliferation rate alterations which occur in the thyroid gland and – assumingly – of the character of ensuing growth processes (hyperplasia, hypertrophy or neoplasmatic growth – benign vs. malignant).

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