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CHANGES IN BCL-2 MARKER EXPRESSION IN THYROID TISSUE UNDER POLYPHARMACY INDUCED BY ANTI-INFLAMMATORY DRUGS

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Abstract

The present study investigated the immunohistochemical expression of the Bcl-2 marker in the thyroid tissue of outbred white rats under conditions of polypharmacy induced by anti-inflammatory drugs. The experimental animals were divided into a control group and groups exposed to combinations of two, three, or four anti-inflammatory drugs. Bcl-2 expression in thyroid tissue samples was determined using the DAB chromogen method, and the resulting microscopic images were subjected to digital analysis using QuPath 0.4.0 software. In the control group, the mean percentage of Bcl-2-positive expression was 4.68%. This value decreased to 3.65% in the group receiving two drugs, to 2.20% in the group receiving three drugs, and to 0.71% following exposure to four drugs. The findings demonstrate a progressive decrease in the anti-apoptotic protection of thyroid cells with an increasing number of anti-inflammatory drugs administered concomitantly. These changes may be used as an immunohistochemical criterion for assessing cellular injury and susceptibility to apoptosis in thyroid tissue under conditions of polypharmacy.

Keywords: thyroid gland, polypharmacy, anti-inflammatory drugs, Bcl-2, immunohistochemistry, apoptosis, thyrocytes, QuPath.

Relevance of the Study

Polypharmacy refers to the simultaneous administration of multiple medications to a patient. Drug-drug interactions arising under such conditions may lead to various adverse morphofunctional changes in different organs and tissues. The combined and prolonged use of anti-inflammatory drugs may affect metabolic processes, proliferative activity, and the mechanisms regulating programmed cell death in thyroid cells.

Bcl-2 (B-cell lymphoma 2) is one of the major anti-apoptotic proteins involved in the regulation of apoptosis. It contributes to the maintenance of mitochondrial outer membrane integrity, limits the release of cytochrome c into the cytoplasm, and prevents activation of the caspase cascade. Preserved Bcl-2 expression supports cell survival, whereas its decreased expression indicates weakening of anti-apoptotic protection and an increased susceptibility of cells to apoptosis.

Determination of changes in Bcl-2 expression in thyroid cells by immunohistochemical methods makes it possible to assess the degree of cellular injury

developing under the influence of pharmacological agents. Therefore, investigating the expression pattern of the Bcl-2 marker in thyroid tissue under conditions of polypharmacy induced by anti-inflammatory drugs is of considerable scientific and practical relevance.

Aim of the Study

To comparatively assess the level of immunohistochemical expression of the Bcl-2 marker in the thyroid tissue of outbred white rats under conditions of polypharmacy induced by two, three, and four anti-inflammatory drugs.

Materials and Methods

A total of 104 outbred white rats were included in the study. The experimental animals were divided into four groups:

- **Group I** — control group (n = 16);
- **Group II** — rats exposed to two anti-inflammatory drugs (n = 26);
- **Group III** — rats administered three anti-inflammatory drugs (n = 30);
- **Group IV** — rats exposed to four anti-inflammatory drugs (n = 32).

At the end of the experiment, the thyroid glands were excised, and tissue samples were prepared according to standard histological procedures. Immunohistochemical examination was performed to determine Bcl-2 expression. The immunopositive reaction was visualized using the DAB chromogen method.

The prepared histological sections were examined at a magnification of 10×10. The obtained digital images were analyzed using QuPath 0.4.0 software. The total number of cells, as well as the numbers of Bcl-2-positive and Bcl-2-negative cells, were determined within the examined areas. The proportion of positive cells relative to the total number of identified cells was calculated as a percentage. The dynamics of changes in Bcl-2 expression were comparatively analyzed among the experimental groups.

Results and Discussion

Immunohistochemical examination of the thyroid tissue of outbred white rats in the control group demonstrated Bcl-2-positive expression ranging from 4.65% to 4.71%, with a mean value of 4.68%. This finding indicates that, under physiological conditions, the anti-apoptotic defense mechanisms of thyroid cells are maintained at a certain level.

In **Group II**, in which polypharmacy was induced using two anti-inflammatory drugs, a total of 658 cells were identified. Of these, 24 cells were Bcl-2-positive, whereas 634 cells were Bcl-2-negative. The percentage of Bcl-2-positive expression was 3.65%, representing a 22.0% decrease compared with the mean value observed in the control group. This change indicates that concomitant administration of two drugs may initiate a decline in the anti-apoptotic defense mechanisms of thyroid cells.

In **Group III**, which received three anti-inflammatory drugs, a total of 866 cells were identified. Among them, 19 cells demonstrated a positive immunohistochemical

reaction, while 847 cells were negative. The percentage of Bcl-2-positive expression was 2.20%. This value was 53.0% lower than that of the control group and 39.7% lower than that observed in the group receiving two drugs. These findings suggest that an increase in the number of administered drugs exerts a progressively stronger effect on the anti-apoptotic mechanisms responsible for maintaining thyroid cell viability.

In **Group IV**, in which polypharmacy was induced using four anti-inflammatory drugs, a total of 1,259 cells were identified. Only 9 cells demonstrated a Bcl-2-positive reaction, whereas the remaining 1,250 cells were negative. The percentage of positive expression was 0.71%. This value was 84.8% lower than that of the control group, 80.5% lower than that of the two-drug group, and 67.7% lower than that of the three-drug group.

The findings demonstrate a consistent decrease in Bcl-2 marker expression in thyroid tissue with an increasing number of anti-inflammatory drugs administered concomitantly. The 4.68% expression observed in the control group decreased to 3.65% following exposure to two drugs, to 2.20% following exposure to three drugs, and to 0.71% following exposure to four drugs.

Such a decrease in Bcl-2 expression may be associated with weakening of the anti-apoptotic defense of thyroid follicular cells and an increased susceptibility to apoptosis. In particular, the marked reduction in the number of Bcl-2-positive cells in the group exposed to four drugs suggests that the combined effect of multiple medications may contribute to the aggravation of dystrophic and atrophic processes in thyroid tissue.

At the same time, decreased Bcl-2 expression represents an indirect indicator of enhanced apoptotic activity. For a comprehensive assessment of the apoptotic process, these findings should be analyzed in conjunction with the expression of Bax, caspase-3, and other pro-apoptotic markers.

Conclusion

1. In the thyroid tissue of outbred white rats in the control group, Bcl-2-positive expression ranged from 4.65% to 4.71%, with a mean value of 4.68%.
2. In the group exposed to polypharmacy with two anti-inflammatory drugs, Bcl-2 expression decreased to 3.65%, representing a 22.0% reduction compared with the control group.
3. In the group receiving three anti-inflammatory drugs, Bcl-2-positive expression was 2.20%, corresponding to a 53.0% decrease compared with the control group.
4. The lowest level of Bcl-2 expression was observed in the group exposed to four anti-inflammatory drugs, where it reached 0.71%. This value was 84.8% lower than that of the control group.
5. An increase in the number of anti-inflammatory drugs administered was associated with a progressive weakening of the anti-apoptotic defense of thyroid cells. Bcl-2 expression may therefore be considered an immunohistochemical

criterion for assessing cellular injury and susceptibility to apoptosis in thyroid tissue under conditions of polypharmacy.

References

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