NEW ANTICANCER SYNTHETIC CARDIOTONIC STEROIDS AND THEIR EFFECTS ON Na,K-ATPase

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The anticancer effect of cardiotonic steroids (CTS) has been observed in several cell lines and it is important to identify new synthesized CTS with anticancer activity. The aim of this work is to investigate the cytotoxic effect of six new synthetic CTS compounds and the relationship of the anticancer effect with the Na,K-ATPase activity. Intact HeLa cells and Na,K-ATPase from rat cerebral hemisphere were treated with synthetic CTS (DGB2, DGB3, DGB4, DGB5, DGB6, DGB7) and digoxin. After 24 hours of cell treatment, we obtained membrane preparation to assess the enzyme activity and expression of Na,K-ATPase α1 subunit by western blotting. The antiproliferative and cytotoxic effect was performed by trypan blue exclusion and MTT assay, respectively. The results demonstrated that IC50 of cerebral Na,K-ATPase inhibition for digoxin was 0.22 ± 0.04 μM. DGB2 and DGB4 showed IC50 of 0.46 ± 0.10 μM and 0.20 ± 0.66 μM, respectively, while DGB3 and DGB5 showed 18 ± 10 μM and 20 ± 7 μM. DGB7 did not show inhibitory effect. In HeLa cells, all the synthetic CTS had cytotoxic effects, but DGB5 showed an IC50 of 0.26 ± 0.06 μM. DGB5 was the only CTS that presented inhibition effect of the Na,K-ATPase activity (150 nM and 10 μM DGB5 caused inhibition of 51 and 96%), but did not modulate the expression levels of Na,K-ATPase α1 subunit. The cell proliferation decreased significantly after 250 nM of DGB5 treatment for 24, 48 and 72 hours. With 96 hours treatment, 10 nM of DGB5 caused reduction of cell proliferation. The only compound which shows correlation between the cytotoxic effect and inhibition of activity of Na,K-ATPase was DGB5. This fact demonstrated that DGB5 cytotoxic effect could be mediated by cellular signaling pathways.

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