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EPIGALLOCATECHIN GALLATE INFLUENCE ON RESTING AND CARBON-FILTERED PLATELETS

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Haemoperfusion on activated carbon adsorbents is successfully used for treatment of acute poisoning, removing the inflammatory cytokines and liver toxins. However, the direct contact of carbon with blood could activate the blood cells, especially platelets resulting in blood clotting. The prospect of current study was to investigate the effect of epigallocatechin gallate (EGCG) on carbon - platelets interaction.

The 1,5 ml carbon columns were pre-adsorbed with EGCG solution and the unbound EGCG was washed by PBS. Human platelet reach plasma (PRP) was filtered through carbon modified with EGCG columns vs. not modified carbon. PRP passed through the columns was collected and platelets granularity, activation and aggregation status were studied using aggregometry, flow cytometry and spectrofluorimetry methods. In spectrofluorimetry study platelets were loaded with pH-sensitive fluorescent dye acridine orange (AO) and the dye release was registered after stimulation of platelets with ADP. The direct effect of free EGCG (0.1 – 0.5 mM) on resting platelets was studied by the same methods. Carbon adsorbent used for PRP filtration was further washed with PBS, fixed with glutaraldehyde and analysed with scanning electron microscopy (SEM).

The flow cytometry analysis showed that EGCG (0.1-0.5 mM) did not affect the shape or granularity of resting platelets but inhibited activation of platelets induced by 0.5 NIH/ml of thrombin and decreased ADP-induced platelet degranulation in spectrofluorymetric study. It was found that pre-incubation of platelets with 0.2 mM of EGCG for 1 min, inhibited platelet aggregation induced by ADP (2,5 μM) or by platelet-activating factor (PAF, 50 ng/ml) by 50 and 75 % respectively.

Although we did not observed any changes in the shape or granularity of platelets filtered through both modified or unmodified carbons, the rate of aggregation of filtered platelets was decreased to 15±4 % in the case of unmodified carbon and to 5±3 % (against 50 % in control) in the case of carbon modified with EGCG. Spectrofluorimetry showed that the accumulation of AO probe was decreased in platelets filtered through unmodified and EGCG-treated carbon by 15 and 50 % respectively. However the subsequent release of AO in response to ADP stimulation was decreased in platelets filtered through the non-modified carbon (by 20%) but increased in platelets filtered through carbon-EGCG (by 25%). According to SEM data the activated and adhered platelets were seen on the non-treated carbon, but no adhered platelets were noticed on the EGCG-modified carbon.

We demonstrated that EGCG was an effective inhibitor of platelet activation and aggregation. Platelets filtered through carbon lost their ability for ADP-induced aggregation. However platelets that were filtered through the carbon pre-treated with EGCG were able to release their granules in respond on ADP stimulation more effective when compared to platelets filtered through unmodified carbon. Thus we can assume that modification with EGCG inhibits platelet reactivity and protects platelets from activation but such platelets remained intact and be able to respond to the stimulus as ADP. This conclusion is supported by SEM images which demonstrated abscence of activated and adhered plateletys on carbon pre-treated with EGCG.

Modification of carbon adsorbents with EGCG can protect platelets from activation and clotting while preserving their functionality during haemoperfusion.

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