Final Report

We investigated the effect of sour cherry seed kernel extract (SCSE) on the size of atherosclerotic plaque, postischemic cardiac function in cholesterol treated rabbits. Our results demonstrated lower plaque size in SCSE treated group compared to the vehicle control animals. We noticed an improved E'/A' ratio after SCSE treatment. Furthermore, we found grater postischemic recovery and smaller infarct size in isolated hearts originated from SCSE-treated animals in comparison with the hearts obtained from vehicle treated animals. Our Western Blot data indicated an enhanced hemeoxygenase-1 (HO-1) and cytochrome c oxidase III (COX-III) expression and COX activity, which could play a role in the cardioprotective effect of SCSE. (Juhasz, Kertesz et al. 2013)

In other experiments we examined the effect of SCSE in a rat model. Rats were treated for 6 weeks and isolated hearts were undertaken to ischemia/reperfusion (I/R). We found superior postischemic functions and reduced infarct size in SCSE-treated group. Furthermore, an enhanced level of HO-1 and Bcl-2 were detected in SCSE treated hearts compared to controls. We found similar pattern in p-Akt/Akt ratio, however it was not significant. (Czompa, Gyongyosi et al. 2014)

The anti-inflammatory property of SCSE were tested lipopolysaccharide (LPS) treated human T cells originated from healthy individuals and patient suffering from rheumatoid arthritis. A dose dependent decrement were seen in CD3+TNF-α+ and CD3+IL8+ immunophenotypes in SCSE treated cells. Furthermore, a dose-dependent enhancement were seen in HO-1 level after SCSE treatment. (Mahmoud, Haines et al. 2014)

Finally, the human safety evaluation of SCSE was carried out. Ten volunteers were assigned into a treated and a control group. The volunteers in treated group received 250 mg of SCSE extract for two weeks once a day. Before and after the treatment blood tests were performed. No sign of any adverse reaction was detected in volunteers on SCSE diet. Furthermore, no significant discrepancy on neither ECGs compared to 0 and 14 days were found. SCSE treated group declared beneficial changes of their quality of life after two weeks especially on their social activity and their general state of health. (Csiki, Papp-Bata et al. 2015)

In conclusion, SCSE possess cardioprotective and anti-inflammatory effect and at the dose we have used is safe in humans.

We studied the effect of different natural products on I/R-induced injury. First, cardiovascular effects of low and high-dose beta-carotene (BC) were studied in a rat model. Hearts obtained from animals treated with low dose of BC exhibited significantly improved cardiac parameters; conversely hearts obtained from high dose fed animals fail to show any significant differences in comparison with the control animals. We have observed similar pattern in case of infarct size, where a significantly reduced infarcted area was detected in low BC group. As BC is a potent antioxidant we studied the total antioxidant capacity of the hearts. As expected, the level of TAC of low BC group was increased, however, in high BC group it remained at the level of control group. In H9c2 cells challenged with H_2O_2 low dose BC enhanced the viability of BC cells, while high doses of BC treatment fail to show any protective effect. Interestingly, our Western blot results revealed that BC induces the level of HO-1 in a dose dependent manner. Our result demonstrated that low doses BC treatment possess cardioprotective effect, while in case of high doses of BC treatment, the protection was diminished. (Csepanyi, Czompa et al. 2015)

In further experiments we tested the hypothesis that *Momordica charantia* (Bitter melon (BM)) extract favorably alters processes in cardiovascular tissue and is systemically relevant to the pathophysiology of type 2 diabetes. Male Lean and Zucker Obese (ZO) rats were gavage-treated with bitter melon (BM) extract. Our results showed that body mass was unaffected by treatment, likewise, peripheral blood fasting glucose levels showed no significant treatment-related effects. However, BM treatment-related improvement was noted in postischemic cardiac functions when Lean, BM-treated animals were compared to vehicle treated Lean control rats. Similar, results was seen in infarct size analysis. Immunohistochemical demonstration of caspase-3 revealed significant correlation between BM treatment and reduced expression of this enzyme in I/R-ed hearts obtained from both Lean and ZO animals. However, BM failed to alter the level of biomarkers related to lipid homeostasis and diabetes in ZO animas. (Czompa, Gyongyosi et al. 2017)

Moreover, we studied the toxicity and mutagenic effect of tarragon. We have performed COMET assay to study the mutagenic effect of tarragon, and after long-term treatment we have studied the level of AST and ALT and the morphology of the liver. Based on our data we can

conclude that in high dose long term treatment of tarragon may have mutagenic effect and liver toxicity. (Kalantari, Galehdari et al. 2013)

We studied the role of alfa-MSH in ischemic-reperfused rat retina. Based on the results of electroretinogrhapic experiments, we can conclude that alfa-MSH is able to protect the retina against I/R-induced injury even if it is administered after ischemia. Moreover, an enhanced level of HO-1 was found in the treated group, indicating that HO-1 could contribute significantly to the retinoprotective effect of alfa-MSH. (Varga, Gesztelyi et al. 2013)

Furthermore, we investigated the effect of alfa-MSH in ischemia/reperfused myocardium. Our results including echocardiographic data, suggests that α-MSH has mild effects on systolic parameters, along with potent antiarrhythmic effects. Moreover, alfa-MSH treatment significantly enhanced the pre and postischemic cardiac functions and decreased the infarct size, which were reversed by Sn-protoporphyrin IX treatment indicating the role of HO-1 in the cardioprotetion induced by alfa-MSH. Furthermore, alfa-MSH treatment induced vasodilation.(Vecsernyes, Szokol et al. 2017)

We investigated the possible connection between autophagy and I/R induced ventricular fibrillation (VF). Isolated mouse hearts were subjected to I/R and divided into two groups based on the development of VF at the onset of reperfusion. Significantly higher levels of Beclin-1 and LC3B-II/LC3B-I ratio were observed in the fibrillated myocardium compared to the nonfibrillated hearts. Interestingly, although Bcl-2 is a major regulator of Beclin-1, level of this protein was not significantly altered. Moreover, Atg7 expression showed a trend, albeit non-significant, towards elevation in fibrillated versus non-fibrillated hearts. Results of the present investigation demonstrate a possible link between reperfusion induced VF and autophagy. (Meyer, Czompa et al. 2013)

We have evaluated the hypothesis that diet-related hypercholesterolemia increases oxidative stress-related burden to cardiovascular tissue. New Zealand white rabbits were divided into four groups, defined as follows: GROUP I, cholesterol-free chow for 12 weeks; GROUP II, cholesterol-free chow, 40 weeks; GROUP III, chow supplemented with 2% cholesterol, 12 weeks; GROUP IV, chow supplemented with 2% cholesterol, 40 weeks. At the 12^{th} and 40^{th} weeks time points echocardiographic measurements were performed followed by sacrifice.

Significant deterioration in major outcome variables measured in the present study were observed only in animals maintained for 40 weeks on 2% cholesterol-supplemented chow. Significant increase in mortality and worsened ejection fraction and general deterioration of cardiac functions, along with increased atherosclerotic plaque formation and infarct size were seen in animals after 40-week of cholesterol treatment. Additionally, myocardium of GROUP IV animals was observed to contain lower levels of HO-1 and COX III protein relative to the controls. Taken together, high systemic cholesterol level enhances the age related cardiac function deteoriation in rabbits. The decreased level of HO-1 and COX III indicated the disturbance in cardiac homeostasis, induced by oxidative stress. (Kertesz, Bombicz et al. 2013) (Kertesz, Bombicz et al. 2013)

Hyperthyroidism elevates cardiovascular mortality by several mechanisms, including increased risk of ischemic heart disease. We have investigated the A_1 receptor reserve in hyperthyroidism. Our results demonstrate that thyroxine treatment does not substantially affect the A1 receptor reserve for the direct negative inotropic effect of adenosine. Consequently, if an agent causing A1 receptor activation is administered for any indication, the most probable adverse effect affecting the heart may be a decrease of atrial contractility in both eu- and hyperthyroid conditions. (Pak, Papp et al. 2014)

We have studied the effect of beta estradiol on hypertrophic responses induced by endothelin-1. In H9c2 cells beta estradiol significantly reduced the endothelin-1 induced hypertrophic responses related to the HO-1 enzyme activity. (Barta et. al. under review)

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