Summary

We have conducted a short term (14 days) human safety evaluation of sour cherry seed extract. A dose of 250 mg of SCSE was consumed for two weeks. Before and after 14-day of SCSE consumption ECG and blood tests were carried out. After the treatment no differences in blood parameters or ECG were found between the SCSE treated and drug-free control group, indicating the SCSE consumption is safe in human. (Csiki et. al. Phytother Res. 2015 Mar;29(3):444-9.)

Reactive oxygen species (ROS) are important determinants of ischemia-reperfusion (I/R)-induced tissue damage. The plant pigment beta-carotene, is one of many phytochemicals with antioxidant properties sufficiently potent to quench ROS activity so as to mediate cytoprotection. Nevertheless, under certain physiological conditions □-carotene may itself exhibit pro-oxidant properties. A demonstration of this effect conducted by our laboratory involved exposure of rats to selected β-carotene doses to evaluate effects on cardiovascular function. Outcomes of these experiments showed that relatively low doses of β-carotene treatment protect I/R-challenged hearts - as evidenced by enhanced postischemic cardiac function and reduced infarct size. Interestingly, high dose treatment failed to improve postischemic cardiac functions. Moreover, our results showed that β-carotene treatment enhanced HO-1 expression in a dose dependent manner, and also improved the antioxidant capacity in animals treated in the low-dose. Further, "*in vitro"* assays revealed that β-carotene protects H9c2 cells challenged with H_2O_2 dose-dependently. The underlying mechanisms of high-dose attenuation of β-carotene-mediated are unknown, but preliminary evidence suggests that features of iron metabolism are contributors. (Csepanyi et, al. Pharmacol Res. 2015 Oct;100:148-56.)

We have tested the effect of β-carotene under diabetic condition. Diabetic animals were treated with various daily doses of beta-carotene for 4 weeks, and then hearts were isolated and subjected to ischemia/reperfusion. Our results show that low dose treatment significantly improved postischemic recovery, which was reflected in decreased infarct size. Interestingly, high dose of beta-carotene failed to protect the heart. Although, beta-carotene treatment increased heme oxygenase-1 expression, we did not observe a better heart function and/or decreased infarct size in case of high dose treatment. Interestingly, beta-carotene improved the glucose tolerance in a dose independent manner. Our results suggest that long-term, low-dose beta-carotene treatment could be effective in the treatment of type-2-diabetes and related cardiovascular diseases. (Csepanyi et. al. Int J Mol Sci. 2018 Mar 28;19(4).)

Effects of the bitter melon extract on diabetic ischemic hearts have been published. Briefly, body mass of both Lean and ZO rats was unaffected by treatment, likewise, peripheral blood fasting glucose levels showed no significant treatment-related effects. We have detected a superior postischemic cardiac function a reduced infarct size in hearts of BM treated nondiabetic animals. A similar trend was observed in diabetic animals, however, it did not reach a significant level. Immunohistochemical demonstration of caspase-3 expression revealed significant correlation between BM treatment and reduced expression of this enzyme in hearts obtained from both Lean and ZO animals. BM extract failed to positively affect T2DM- and

cardiovascular-related outcomes at a level suggesting use as a standalone treatment. Nevertheless, the encouraging effects of BM in the enhancement of cardiac function, suppression of post-ischemic/reperfused infarct size extent and capacity to modulate serum cholesterol, will likely make it useful as an adjuvant therapy for the management of T2DM and related cardiovascular diseases. (Czompa et. al.Molecules. 2017 Mar 20;22(3). pii: E488)

Furthermore, the comparison between the effect of raw and black garlic on postischemic cardiac function was studied. We have performed a GC-MS analysis to monitor the alteration in compounds during the ageing process. Male rats were feed for one month with fresh and aged black garlic. At the end, isolated hearts were undertaken to I/R. We have detected enhanced postischemic recovery in both treated animals compared to the control animals. However, no significant differences between the two garlic treated group were observed indicating that the cardioprotective ability of garlic remains intact during ageing process. At molecular level, we have detected enhanced level of HO-1 were seen in fresh and aged black garlic after I/R. Furthermore, aged black garlic treatment prevented I/R induced iNOS reduction. (Czompa et. al Int J Mol Sci. 2018 Mar 28;19(4).)

We have studied**.** **r**adical scavenging activity (ABTS), oxygen radical absorption capacity (ORAC) and ferric reducing the antioxidant properties and oxidative transformation of nine chromone derivatives selected from the molecule bank of the University of Debrecen antioxidant power (FRAP) were studied. Furthermore, MTT assay was carried out to test the cytotoxic effects of the compounds. Each compound showed a significant ORAC value compared to the reference. However, the compound 865 possess superior FRAP and ABTS activity in comparison with the reference and other tested molecules, respectively. The oxidative metabolism of compound 965 was studied in vitro. The molecule was oxidized by the Fenton reaction, artificial porphyrin and electrochemistry; then, mass spectrometry was employed to analyse the metabolites. Four possible metabolites were detected. The results revealed the compound 865 to possess good antioxidant properties and to be stable metabolically; hence, it is worth investigating its effects in vivo. (Csepanyi et. al. Molecules. 2017 Apr 6;22(4). pii: E588)

We have evaluated the ability of beta estradiol (□-E) to alter ET-1-associated hypertrophic activity and decreased expression of heme oxygenase-1 (HO-1) in H9c2 rat cardiomyoblast. H9c2 cells were stimulated with ET-1 and evaluated for changes in cell size, cell viability and expression of the cytoprotective heat shock protein HO-1, with □-E included in selected cultures to evaluate its effect on ET-1-mediated changes. The application of ET-1 significantly increased the average cell size and decreased the cell viability and HO-1 protein content. Moreover, □-E was observed to significantly counteract these effects in cardiomyoblasts stimulated with ET-1. These effects were associated with a restoration of HO-1 protein content and expression under both in vitro and in vivo conditions. (Bartha et. al. Naunyn Schmiedebergs Arch Pharmacol. 2018 Apr;391(4):371-383.)

Molecular mechanisms underlying doxorubicin cardiotoxicity are still being investigated, but known to involve oxidative stress, mitochondrial dysfunction and the dysregulation of autophagy. We examined the protective role of metformin and its effect on autophagy in doxorubicin-induced cardiotoxicity. Sprague-Dawley rats were segregated into four groups at random. The doxorubicin-treated group rats received doxorubicin (3 mg/kg every second day) intraperitoneally. The metformin-treated group received 250 mg/kg/day metformin via gavage. The doxorubicin + metformin-treated group received both at the above-mentioned doses. The control group received vehicle only. Following the two-week treatment, the hearts

were isolated, and cardiac functions were registered. Serum levels of lactate dehydrogenase (LDH), creatine kinase iso-enzyme MB (CK-MB) enzyme, Troponin-T, and cardiac malondialdehyde (MDA) were also measured. Heart tissue samples were histopathologically examined by using Masson's trichrome staining, and Western blot analysis was conducted for evaluating the expression level of AMP-activated protein kinase (AMPK) and autophagy-associated proteins beclin-1, LC3B-II and p62 respectively. The result revealed that treatment of metformin produced an increased cardiac protection against the development of cardiotoxicity manifested by a significant decrease in serum Troponin-T and cardiac MDA levels, and remarkable improvement in the heart function in connections with histopathological features. Furthermore, by focusing on the contribution of autophagic proteins, it was found that metformin normalized autophagy, which may help cardiomyocytes to survive doxorubicin-induced toxicity. These results suggest the use of metformin, which would be a preferable drug for patients receiving doxorubicin. (Zilinyi et. al. Molecules 2nd round of review is ongoing)

Moreover, we have studied the heart tissue responses to prolonged hypoxia or hyperoxia, especially how such situations might lead to the activation of survival mechanisms or to trigger cell death. Seven-week-old Foxn1 mice were exposed to hypoxia (10% O_2), normoxia (21% O_2) or hyperoxia (30% O_2) for 28 days, then the heart tissue were excised and analyzed. The alterations in redox balance, housekeeping protein levels, autophagic and apoptotic process regulation were studied. The level of hypoxia inducible factor-1 (HIF-1α) was increased by hypoxia while HIF-2α was not affected by treatments. The altered O_2 fractions in breathed air inversely elevated the housekeeping protein levels, the oxidative stress and autophagy. Surprisingly, our results revealed alterations in the level of housekeeping proteins. The expression of α-tubulin, actin and GAPDH were increased in the hypoxic while were decreased in the hyperoxic group. Chronic hypoxia activated biochemical markers of autophagy, we observed elevated levels of Beclin-1 but LC3B-II and p62 were constant. Nevertheless, we measured significantly enhanced a number of Tunnel positive cells and higher Bax/Bcl2 ratio by hyperoxia with respect to hypoxia. (Gyongyosi et. al. Oxidative Medicine and Cellular Longevity pending review)

It has been shown that pacing-induced VF results in damage by reactive oxygen species that exacerbate cardiac arrhythmogenesis and many other adverse effects. Our investigation builds on the results of these and related studies to evaluate the effect of electrically stimulated VF on cardiac functions in isolated, Langendorff apparatus-mounted isolated, working rat hearts. Each group of hearts was subjected to 0 (Control), 1, 3 or 10 minutes of VF, followed by selected recovery periods and evaluated for cardiac functions, including aortic flow (AF), coronary flow (CF), cardiac output (CO), stroke volume (SV); and heart rate (HR). Hearts were also evaluated for VF effects on infarcted zone magnitude – and Western blot analysis was conducted on heart tissue for expression of the apoptotic biomarker cleaved-caspase3 and the autophagy- and senescence-associated proteins: p62, mTOR phosphorylated mTOR (P-mTOR), LC3B-II and Atg5-12 complex. Analysis of data shown here revealed that VF induced degradation in cardiac function that prominently included variable post-VF capacity for recovery of normal rhythm from heart to heart; increased extent of infarcted heart tissue; altered expression of cleaved caspase-3 suggesting the potential for VF-mediated amplification of apoptosis. VF influence on the expression of p62, LC3B-II and Atg5-12 proteins was complex, possibly due to differential effects of VF-induced reactive oxygen species on the expression of the many component proteins comprising the autophagic program. VF was observed cause time-dependent fluctuations in autophagy, which with additional analysis in ongoing investigations, are likely to yield novel therapeutic targets for the management of arrhythmias.

(Czeglédi et. al.)

We investigated the autophagic process in H9c2 cells in response to different concentration of AT-II treatment. To induce hypertrophy H9c2 cells were exposed to different amount of AT-II as follows: I: 100 nM, II: 400 nM, III: 1000 nM, IV: 10000 nM, V. control. We have examined the cell viability via MTT assay. After FITC-Phalloidin staining, we examined the alteration of cell size. To visualize autophagic vacuoles Cyto-ID staining were carried out. Furthermore, the expression levels of autophagic protein such as Beclin-1 and LC3B-II were evaluated by Western blot. A slight decrement was detected in cell viability in IV. group. Based on our microscopy experiments the cell size was significantly (approximately 15-20 %) greater in the treated groups indicating the hypertrophy, the most intense alteration occurred in IV. group. Cyto-ID staining showed an increasing fluorescence signal in the treated groups. In our Western Blot results, an enhanced LC3B-II and Beclin-1 levels were found in response to the lower AT-II treatment. Taken together our results suggest that in case of the AT-II inducted hypertrophy the autophagy is increasing, which may help to reduce damaged protein organelles.

The klotho gene was identified in 1997. It functions as an aging suppressor gene in mammals. The anti-ageing protein produced in several tissues but predominantly in the kidney. Several studies show that autophagy plays an important role in aging. Numerous studies revealed that autophagy decreases with aging. In this study our aim was to investigate the connection between autophagic process and the level of klotho protein. Aged C57BL/6 mice (24 months old) were treated with intraperitoneal injection of rapamycin (1.5mg/bwkg) weekly for 12 weeks to induce klotho protein. We have monitored the body weight during the treatment. Thereafter the organs were isolated. The expression levels of anti-aging klotho protein and autophagic proteins such as LC3B-II and p62 were evaluated by Western blot. We have also measured the level of serum cytokines. Furthermore, we have visualized the immunohistochemical localization of klotho protein in the different organs. Furthermore, we have measured the active and passive force and Ca^{++} sensitivity in isolated permeabilized cardiomyocytes. We have noticed decreased lifespan in the control group compared to mice received rapamycin, where the prolonged lifespan suggests that klotho functions as an anti-aging protein. We have found an increased expression level of the klotho in most of the organs, especially in the kidney, brain and liver. Furthermore, a decreased p62 protein level was found in the treated group, which is involved in the regulation of apoptosis and autophagy. We found enhanced Ca^{++} sensitivity in myocytes originated from rapamycin treated animals. Taken together our results suggest that rapamycin induced klotho protein expression is accompanied by enhanced autophagy, which may help to reduce damaged proteins and organelles.

We have examined the possible connection between the two systems. H9c2 cardiomyoblast cells were treated with different dose of hemin or cobalt-protoporphyrin IX (CoPPIX) or vehicle (20mM NaOH solution) for 24-h. Moreover, we have tested that CO-exposure may alter the level of autophagic proteins. Rats were exposed to normal air supplied with 100 ppm CO twice a day for one month. After the treatment of the cells cytotoxicity was measured by MTT assay. Furthermore, staining was carried out to determine the alterations in cell size. To study the autophagic process CytoID staining was carried out and cells were studied by fluorescence microscope. On the other hand, Tunel assay was performed and determined the level of DNA fragmentation. Moreover, Western blot analysis was performed to analyze the level of HO-1,

certain autophagy related proteins (Beclin-1, LC3B-II, p62) and Caspase-3 as apoptosis marker.

We have detected a slight decrement in cell viability in the hemin and CoPPIX treated groups in a dose dependent manner. The cell size did not alter. As it was expected a robust induction of HO-1 were detected with both of inducer. Beclin-1 expression was unmodified by both of inducer. An enhanced number of autophagosome were detected by CytoID staining, and elevated level of LC3B-II was found in the highest hemin and CoPPIX treated groups. Surprisingly, the level of p62 was also enhanced in the same groups, which may show an uncomplete autophagy. Additionally, pro-caspase-3 were significantly decreased, which strongly suggest the activation of apoptotic pathway. Our preliminary results indicated an elevated level of HO-1 and LC3-II in hearts originated from CO-treated animals. Taken together, our results show that, there is a connection between HO-1/CO system and autophagy process, but further experiments need to be carried out to precisely understand the nature of the connection.

We have studied the effect of capsaicin on coronary arteries in isolated mouse hearts, and on ischemia reperfusion in isolated rat hearts. Isolated mouse hearts were perfused with different doses of capsaicin (10^{-8}-10^{-5} M) and coronary flow alteration was measured. After washing out capsaicin the same protocol was repeated. Isolated working rat hearts were perfused with capsaicin (10^{-6} M) and undertaken to I/R. Heart functions and infarct size were studied. Our results indicates that capsaicin has a vasoconstrictive effect in mouse hearts in a dose from 10^{-6} M, which effect can be washed out. Moreover, capsaicin treatment significantly reduced infarct size indicating its cardioprotective effect in rat hearts.

Claudins are tetraspan transmembrane proteins of tight junctions. Members of this protein family are expressed in a tissue specific combination resulting in tissue specific barrier characteristics. According to latest research outcomes, there are 7 claudins mainly myocardial origin. Claudin-12 is highly abundant in the myocardium especially in the lateral membrane and regulates other claudins expression, however, the functions of claudin-12 are not clear. By our experimental arrangement, we have aimed to study the susceptibility of claudin-12 knock out mice hearts to ischaemic/reperfusional injury compared to wild type mice hearts. Under deep anesthesia thoracotomy was performed, followed by excision of hearts which were then mounted on isolated working heart system. After 10 minutes of working perfusion, 20-minute global ischemia was initiated followed by 120-minute reperfusion. Functional parameters including heart rate, aortic flow, coronary flow and aortic pressure were measured during the performed protocol. Heart weight/tibia length ratio was also measured to detect possible signs of cardiac hypertrophy We noticed a slight reduction in pump functions in claudin-12 KO hearts compared to wild type controls. Furthermore, a slight increment in heart weight/ tibia length ratio in claudin-12 KO animals suggesting the development of myocardial hypertrophy.

Ischemic heart conditions are among the main causes of death worldwide. One of the strategies of avoiding myocardial infarction is the low-dose, prophylactic use of acetylsalicylic acid (ASA), an inhibitor of platelet aggregation. To avoid the gastrointestinal damage, ASA prodrugs bearing nitric oxide (NO)-donating moiety covalently conjugated to ASA have been synthesized and evaluated extensively worldwide. Herein the synthesis of a new hybrid ASA ester covalently attached to the NO donor linsidomine, an active metabolite of molsidomine (MOL) is reported. Cell viability assay and hemolysis tests were performed in H9c2 cells and rat erythrocytes, respectively. Our new compound, the **ERJ-500** not affected negatively the viability of living cells in the concentration range of 100 nM to 100 μM. Using the *ex vivo* Langendorff method on hearts originated from female rats, compound **ERJ-500** displayed a

dose-dependent, outwashable vasodilative effect in coronary arteries. Based on these observations it can be expected that our new hybrid ASA may contribute to new approaches in the therapy of ischemic heart conditions and associated syndromes. (Czompa et. al. Eur. J. Pharm. pending review)