Report of the pilot-study done for the proposed investigation on the possible synergic effect between high dose ascorbic acid application and oncothermia treatment

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Abstract
According to recent investigations, the parenteral application of ascorbic acid (vitamin C) at high doses has significant antitumor activity in in vitro assays. This fact is a very important using ascorbic acid as complementary drug with standard antitumoral therapy or in cases where currently no other potent treatment is possible. Although the specific method of action is still unclear: high concentration of ascorbic acid produces oxidative shock by H2O2 lethal for tumor cells beyond a certain level, however healthy cells can survive the same stress effect. The goal of our experiment was to determine the possible potentiating effect of application of high dose pH-neutralized ascorbic acid to the normal oncothermia treatment method. The NMRI mice were inoculated with C26 Murine Colon Carcinoma cell line subcutaneously at both of their femoral regions and kept till the tumors reach symmetrically the 10 mm in diameter. Four experimental groups were made, containing 4 female animals in each. The formed groups of animals were: Gr1. no treatment (control), Gr2. only ascorbic acid treatment, Gr3. only oncothermia treatment, Gr4. both ascorbic acid and oncothermia treatment. Both vitamin-C and oncothermia treatment were applied once (“single-shut” treatment regime), ascorbic acid was pH-neutralized and applied intra-peritoneal in dose of 2 g/kg bodyweight. Oncothermia treatment was applied only to the right limb tumor, the other side was used as internal control. After the treatment the animals were sacrificed, all tumors were removed and analyzed histopathologically. The other organs were routinely checked as well. Our main question centered on the comparison of the cell-destruction ratio of the various applied treatment regimes, and study the possible synergy or additive crosspotentiating of the methods. In this pilot study results are showing that because of some unknown reasons, high dose ascorbic acid application showed no synergic or adjuvant effect with OTM therapy, even the combination decreased the effectivity of the OTM compared to the results of monotherapy. In the proposed, large number animal experiment results of this study should be considered.

Introduction
According to recent investigations, the parenteral application of ascorbic acid (vitamin C) at high doses has significant antitumor activity in in vitro assays. This fact is a very important using ascorbic acid as complementary drug with standard antitumoral therapy or in cases where currently no other potent treatment is possible. Although the beneficial effect of the ascorbic acid in anti-neoplastic therapy has some controversial reports in the literature [1-3] and the specific method of action is still unclear: high concentration of ascorbic acid produces oxidative shock by H2O2 lethal for tumor cells beyond a certain level, however healthy cells can survive the same stress effect [4]. As for the application, it was reported that intravenous ascorbic acid treatment is much more efficient, since this way more than 70-fold higher plasma concentration is elucidable than in case of oral application [5]. To achieve proper effect, high plasma level of ascorbic acid is required, so in human cases intravenous dosages are considered between 0,15 to 1,5 g/kg doses [6, 7].

Objective
The goal of our experiment was be to determine the possible potentiating effect of application of high dose pH-neutralized ascorbic acid to the normal oncothermia treatment method. The dose to use we considered to be 2 g/kg accordingly to human trials [7] and our intra-peritoneal acute toxicity test (not reported). We choose the intra-peritoneal application because the absorption from abdominal cavity is very fast and complete, nearly equal to intravenous application and it is much easier performable in mouse than IV application. However in this case only not irritative materials can be applied, so neutralization of ascorbic acid by sodium-hydroxide is necessary.

Method
The NMRI mice intended to inoculate with C26 Murine Colon Carcinoma cell line subcutaneously at both of their femoral regions and kept till the tumors reach symmetrically the 10 mm in diameter. C26 cells were be cultivated in RPMI 1640 Glutamax medium (Invitrogen, Carlsbad, California, USA). Inoculation was be done by 7500000 cell/ml concentration liquid cell suspension, using 0,1 ml
subcutaneously each side. Incubation time for tumor growth is expected to be around two weeks. Vitamin C solution of 1M in concentration was be created by using dry ascorbic acid (Sigma Aldrich, St. Louis, Missouri, USA) and sterilized purified water, and was be neutralized by 10M sodium-hydroxide solution (Sigma Aldrich, St. Louis, Missouri, USA). We created four experimental groups, containing 4 female animals in each. The formed groups of animals were: Gr1. no treatment (control), Gr2. only ascorbic acid treatment, Gr3. only oncothermia (OTM) treatment, Gr4. both ascorbic acid and oncothermia treatment. Both vitamin-C and oncothermia treatment was applied once (“single-shut” treatment regime), ascorbic acid was pH-neutralized and applied intra-peritoneal in dose of 2 g/kg bodyweight. Oncothermia treatment was applied only to the right limb tumor, the other side was be used as internal control, the incubation period between vitamin C application and OTM treatment was 30 minutes. The animals were sacrificed 24 hours after the treatment, all tumors were removed and analyzed histopatologicaly. The other organs were routinely checked as well.

**Results**

In our pilot-study, we experienced that vitamin C as monotherapy did not do any change in tumor remission compared to the control samples. As for OTM as monotherapy, the treated side tumor showed greater dead tissue area than the both of untreated side and control animals, same level as experienced in earlier studies. The combinational therapy showed controversial result, as the dead tissue amount in the tumors was the same in both treated and untreated sides, and it was smaller than in case of OTM monotherapy at the treated side.

**Conclusion**

In this pilot study results are showing that because of some unknown reasons, high dose ascorbic acid application showed no synergic or adjuvant effect with OTM therapy, even the combination decreased the effectivity of the OTM compared to the results of monotherapy.

In the proposed, large number animal experiment results of this study should be considered. It is highly probable that “single shut” ascorbic acid application will not be appropriate to achieve any synergic effect. Also, incubation time between vitamin C application and OTM treatment should be greater than the applied 30 minutes.

According the results we have got in our experiment suggest us that we should turn our interest from ascorbic acid to other possible materials as potentiating agent for oncothermia treatment in the future.

**References**