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In vivo behavior of carboxymethylcellulose based microgels containing ^{67}Cu

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S1. ***Separation of copper radionuclides***

The zinc target irradiated by bremsstrahlung photons with E= 55 MeV on a split microtron dissolved in 36% HCl and separated by ion-exchange chromatography on a column with Cu-resin sorbent (3 ml), where copper sorbed at pH 2.4, and zinc washed off the column. After the zinc has been washed out, copper desorbed with HCl (5 mole L^{-1}). For working with mice, it is used solutions in which the activity of ^{64}Cu was already insignificant, since $T_{1/2}$ (^{64}Cu) = 12.7 and $T_{1/2}$ (^{67}Cu) = 61.8 h, respectively. The distribution of activity monitored on a gamma spectrometer with a semiconductor detector "Canberra GC 3020" (USA). The technique described in detail in [7]. On average, the ^{65}Zn/^{67}Cu ratio after elution of zinc with a 0.01M hydrochloric acid solution was 570. No radioactive zinc detected after copper elution. The total activity of copper was 44.7 kBq, which was sufficient for laboratory tests. Radioactive copper solution $^{67}CuCl_2$ used to prepare CMC-^{67}Cu microgels and inject into mice.

S2. ***Thin layer chromatography (TLC).***

The silufol plates and the elution system: butanol: acetone: formic acid = 1: 1: 1 with the development of a chromatogram by iodine vapor for CMC identification (R_f = 0.68) used for TLC. Determination of the radiochemical purity of microgel preparations (92%) carried out by autoradiography.

S3. ***CMC-Cu^{2+} binary complexes.***

The preparation of the initial CMC microgels and binary systems CMC-Cu^{2+} for labeling with tritium described in detail in [10]. The following parameters of the samples used in the work: [Cu^{2+}] / [CMC monomer unit] = 0.11 ± 0.01; hydrodynamic diameter 370 ± 12 nm; EPM = - (2.9 ± 0.01) (μm/s) / (V/cm).

S4. ***Preparation of CMC-$^{67}Cu^{2+}$ microgels.***

In two test tubes, 0.5 ml of $^{67}CuCl_2$ solutions containing 20 and 10 pg of radioactive copper, respectively, were prepared. Gamma spectrometry was used to determine copper. Both solutions were added dropwise to 0.5 ml of CMC solution (M = 90 kDa, degree of substitution 0.7, Sigma Aldrich, USA) with a concentration of 1 ng ml^{-1}. The solutions were for 12 h on a mechanical

shaker. The resulting CMC-^{67}Cu microgels had a Cu/mono CMC ratio of 1/15 and 1/7, respectively.

S5. The aqueous solutions of the preparations evenly distributed on the walls of the reaction vessel, quickly frozen with liquid nitrogen, and the water removed by lyophilization under vacuum. The mass of the supported substance was 0.74 and 0.86 mg for CMC and CMC-Cu, respectively. The vessel containing target connected to a vacuum setup for working with tritium, evacuated to a pressure of less than 0.01 Pa with a foreline pump, the system filled with gaseous tritium to a pressure of 0.5 Pa. The reaction activated by heating the tungsten coil to 1860 K for 10 s, while the walls cooled with liquid nitrogen. The resulting preparation dissolved in water and transferred to a glass flask. The labeled preparations dissolved in water, kept for 1 day, and evaporated to dryness on a rotary evaporator. In the next purification step, dialysis through 2 kDa MWCO membranes (OrDial D-Clean) was used. Dialysis performed against 1 L of water for 4-5 days. The external solution changed periodically. The preparations dissolved in water, placed in dialysis bags against 1 L of water, and kept at 4 ° C for 4 days. (CMC-Cu) and 5 days. (CMC). The radioactivity of the external solution measured periodically and the solution replaced with a new one. The change in the radioactivity of the external solution showed that the removal of low-molecular-weight impurities and tritium from the labile positions of the molecules occurred in the first 2 days dialysis (Fig. S1). The purified preparations transferred into sterile vials and stored at 4°C. The specific radioactivity of the preparations was 1.28 and 0.95 mCi mg^{-1} CMC and CMC-Cu, respectively. At all stages of this work, the radioactivity of solutions measured using a RackBeta 1215 liquid scintillation spectrometer (LKB, Finland) using an Ultima Gold scintillation liquid (PerkinElmer, USA). To determine the registration efficiency, we used calibration curves obtained using tritium radioactive standards (PerkinElmer, USA).

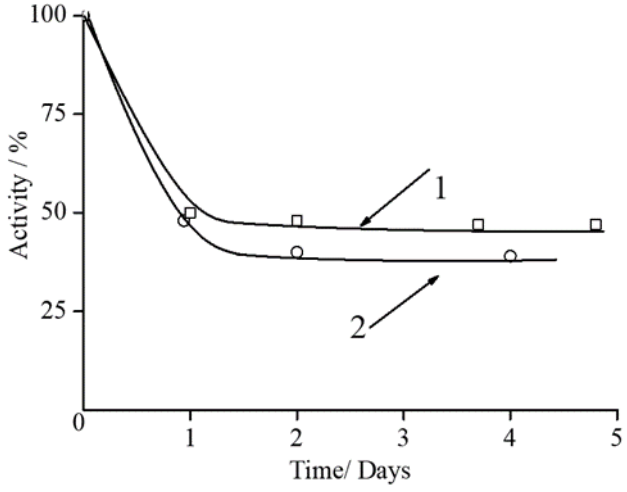

Figure S1 Changes in preparation activity during dialysis cleaning: *[^{3}H]*CMC (1) and *[^{3}H]*CMC-Cu (2).

S6. Aqueous solutions of *[^{3}H]*CMC and *[^{3}H]*CMC-Cu evaporated to dryness and dissolved in 0.15 M NaCl to obtain a final concentration of 0.08 mg/ml. The kinetics of drug distribution in organs assessed in 24 male mice (4 mice per point). The drugs injected into the tail vein. After a certain time, the mice euthanized by the method of cervical dislocation, after which blood, urine, and individual organs collected. A vein was also removed from the tail, if possible, to assess the activity of the drug remaining at the injection site. The organs weighed and the solvent SOLVABLE (SigmaAldrich, USA) added in a ratio of 1:10 of the organ weight. The liver was frozen, homogenized by grinding in a porcelain cup with a mortar, and a portion sufficient for reliable determination of the radioactivity of tritium and taken for dissolution in SOLVABLE. One ml of SOLVABLE added to blood samples and decolorized by adding 30% H_2O_2. The resulting mixtures heated at 60°C in an ultrasonic bath Grad (Russia) from 1 to 6 h with periodic switching on of ultrasound (220 W) for 10 min until the tissues were completely dissolved.

The radioactivity of dissolved organs measured by mixing 0.2 ml of the solution with 10 ml of Ultima Gold scintillation liquid (SigmaAldrich, USA). The urine mixed directly with the scintillator without prior preparation. The vials ready for measurement kept for at least 1 h and then measured on the YSS TriCarb 2810 TR (PerkinElmer, United States). Registration efficiency determined by the spectral parameter tSIE. To check the correctness of the application of the standard calibration of the spectrometer, we used the internal standard method, i.e. after measuring radioactivity, some vials filled with a radioactive solution with a known activity.

The radioactivity of the organ, reflecting the content of CMC in it, calculated from the counting rate of the vials, taking into account the efficiency of tritium registration and the fraction of the solution taken for measurement.

$$\varepsilon = (C_{s+i} - C_s) / D$$

where C_{s+i} is the counting rate of the sample with the addition of a standard, C_s is the counting rate of the sample without the standard, D is the activity of the standard.

The distribution of CMC by organs compared using the formula:

$$\delta\ (\%) = A_x / [m_x (A_{int} - A_{vein})]$$

where δ (%) is the specific content of CMC in the organ, normalized to the total amount that entered the animal A_x; m_x is the radioactivity of the organ and its mass; A_{int}, A_{vein} is the radioactivity introduced into the animal and its part remaining in the tail vein, respectively.

The average value of the specific content of CMC in organs found from the experimental data for four animals.

S7. UV spectrometry was used to determine copper. A standard concentration of solutions of 1 mg ml^{-1} used. After irradiation, the samples transferred into dialysis bags (Sigma, MWCO ~ 12 kDa)

and incubated 48 hours against 50 ml of distilled water. The content of copper released from the gel structure estimated: 5 ml aliquots taken from the external solution after dialysis and the copper content determined by the ICP-AES method on an IRIS Intrepid II DUO ICP-AES spectrometer (Thermo Electron, USA) with a semiconductor CID detector. Copper determined at a wavelength of 324.7 nm.

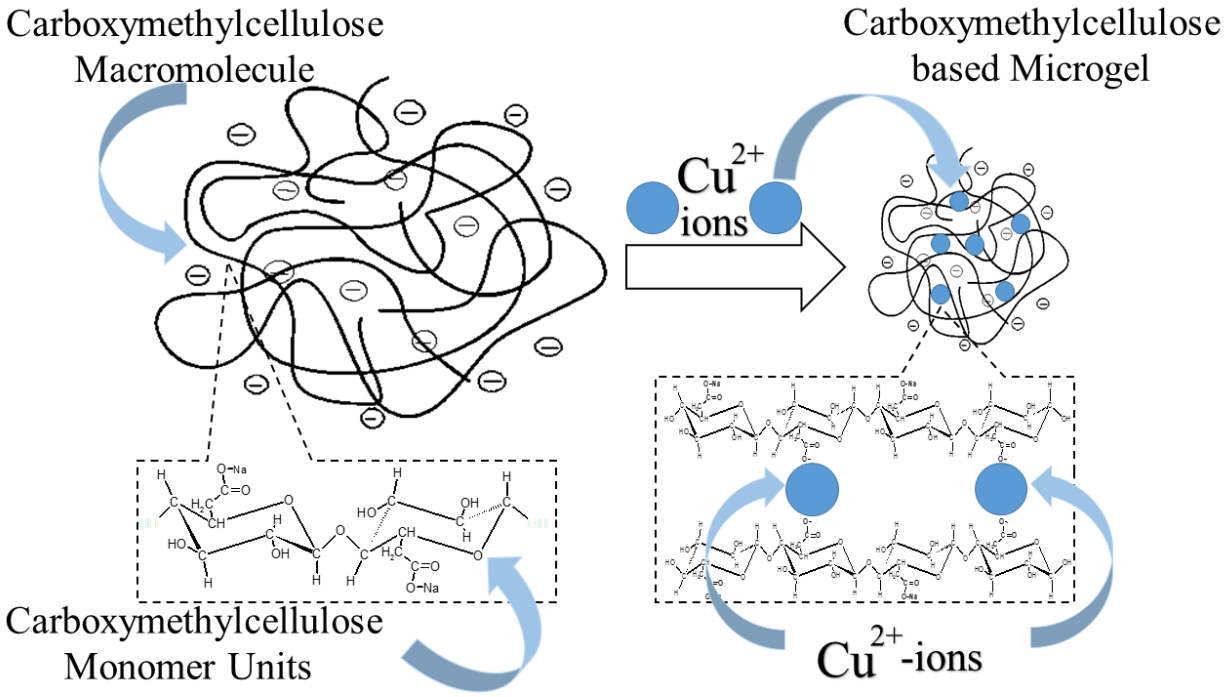

Figure S2 Scheme of CMC microgel cross-linked with copper ions.