SULFADIAZINE-^{35}S AS A POTENTIAL RADIOPHARMACEUTICAL DRUG

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Malignant neoplasms are currently treated with open radionuclides in conjunction with radiopharmaceutical drugs, usually colloidal gold, ^{131}I, or ^{32}P [6, 11]. There have been reports in the specialist literature of the experimental use of ^{125}I [10], ^{51}Cr [5], and ^{60}Co [7] as radionuclide tags, together with drugs tagged with ^{67}Ga, ^{74}As, ^{124}Sb, and ^{75}Se [9]. The applications of such radionuclides are however, restricted. Most of them show insufficiently high selective concentrations in the tumor cells under conditions in which undesirably high concentrations are not found in the vital organs, and in addition these drugs are sources of γ- or hard β-rays, resulting in irradiation of the surrounding healthy tissues. For these reasons, the search for radiopharmaceutical drugs remains of great importance.

The object of the present investigation was to synthesize a drug whose physicochemical properties should give rise to optimum concentrations in the affected area to obtain therapeutic absorbed doses at low levels of accumulation in other organs and tissues, including the vital organs. The search for a drug was carried out amongst organic compounds with specific properties (biological activity, and tropicity for tumor tissues). Preliminary studies showed sulfadiazine, a sulfanilamide derivative, to apparently be promising in these respects. It is known from the literature [4] that it accumulates in transplanted tumors to 3-4 times the levels in the vital organs (bone marrow and liver). The radionuclide chosen for incorporation into sulfadiazine was ^{35}S, which emits soft β-radiation, and does not have high tropicity for organs and tissues. It has been shown in experimental and clinical studies [2, 3] that the direct lymph vessel introduction of antitumor drugs, including radionuclide drugs, increases their therapeutic effectiveness and reduces generalized toxic effects. This mode of introduction of sulfadiazine-^{35}S was used by us when examining its pharmacokinetics in animals with transplanted and lymph node tumors.

Sulfadiazine-^{35}S was obtained as described in [8]. The starting material for the synthesis was N-acetylsulfanilyl chloride-^{35}S (III), obtained from N-acetylsulfanilic acid-^{35}S and phosphorus pentachloride. Reaction of (III) with 2-aminopyrimidine gave N^4-acetyl-N^1-2-pyrimidinylsulfanilamide-^{35}S (IV), which was hydrolyzed with 6% sodium hydroxide to give sodium sulfadiazine-^{35}S (V), which gave sulfadiazine-^{35}S (VI) on treatment with hydrochloric acid.

The purity of (VI) and its identity with an authentic, pure sample was checked by TLC together with autoradiography on Silufol plates in the system ethyl acetate–methanol–25% ammonia. The chromatograms and radioautochromatograms showed the presence of only one component in this material, namely sulfadiazine-^{35}S with R_f 0.3. The specific radioactivity was 180 MBq/g, and the total radioactivity 170 MBq.

Examinations of the distribution of the sulfadiazine-^{35}S were carried out in 20 chinchilla rabbits weighing 2-3 kg, with Brown-Pierce tumors transplanted into the popliteal lymph node, and in intact rabbits, by direct administration into the lymph vessel. The drug was administered as a suspension in physiological saline. The results of the studies of the pharmacokinetics of the drug in intact animals and tumor-bearing animals are shown in Table 1. In examining the data for the accumulation of the drug in intact and damaged lymph nodes, it is necessary to bear in mind the differences in the weights of the lymph nodes. Lymphatic nodes damaged by the tumor weighed 1-2 g, while those in intact animals weighed 0.2-0.4 g.

It will be seen from Table 1 that two hours after administration, one third of the drug

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TABLE 1. Distribution of Sulfadiazine-^{35}S (as % of the activity introduced per g of body weight) in the Organs and Tissues of Rabbits with Tumors and Intact Rabbits Following Direct Lymph Vessel Administration (M ± m)

Organs and tissues	Time from administration of drug			
	2 h		24 h	
	intact	tumor-bearing	intact	tumor-bearing
Regional lymph node	116,15±4,70	35,45±8,3	111,10±8,30	31,73±3,10
Lumbar lymph node	0,023	0,01	0,01	0,01
Control lymph node	0,01	0,006±0,002	0,003	0,005±0,001
Bone marrow	0,005±0,001	0,003	—	—
Liver	0,004±0,001	0,007±0,001	—	—
Spleen	0,015±0,003	0,01	—	—
Kidneys	0,006±0,002	0,01	0,007	0,004±0,001
Lungs	0,006±0,001	0,01	0,004	0,002
Muscle	0,006	0,01		
Blood	0,012±0,001	0,02	0,002	0,001

introduced had accumulated in the tumor lymph nodes per gram weight. This value is statistically significantly less by a factor of 3.3 ($P < 0.001$) than in the intact regional lymph nodes. Following direct lymph vessel administration in tumor-bearing animals, one day after administration the specific amounts present in the lymph nodes were also significantly less by a factor of 3.5 ($P < 0.001$) than in intact animals. In the other organs and tissues, including the bone marrow, spleen and liver, the radioactivity per 1 g weight did not exceed one percent of the amount introduced. Subsequently, the amounts of the drug decreased steadily. Over the next five days, the specific concentration of sulfadiazine-^{35}S in the tumor-bearing lymph nodes decreased by a factor of 18, and intact nodes, by 17.5%, and the count rates for the organs and tissues did not exceed background levels.

It is noteworthy that in the first 24 hours following administration the specific concentrations of the drug in the regional lymph nodes, both in the intact and tumor-bearing animals, remained virtually unchanged. This observation is of particular value, since we are contemplating a radiopharmaceutical drug for therapeutic purposes in primary and secondary tumor-damaged lymph nodes.

We have synthesized sulfadiazine-^{35}S, the biological behavior of which on direct lymph vessel administration to tumor-bearing animals gives high levels of accumulation in transplanted tumors of the regional lymph nodes. The drug remains in these nodes for extended periods of time, thus enabling various absorbed doses to be given depending on the precise object of treatment with the drug. Further, the levels of accumulation attained in the organs and tissues are extremely low, which is of considerable importance in respect to experimental and clinical radiobiology.

EXPERIMENTAL (CHEMISTRY)

N-Acetylsulfanilic Acid-^{35}S (II). To 2.03 ml (38.1 mmole) of sulfuric acid-^{35}S was added slowly with cooling 4 ml of acetic anhydride. Acetanilide (2 g, 14.8 mmole) was added in small portions with stirring, and the mixture heated on the water bath to 93-100°C for 30 min. The mixture was cooled in an ice bath, diluted with 10 ml of cold acetone, and filtered. The product was washed with a small amount of acetone and dried at 90°C to give 3 g (95%) of (II).

N-Acetylsulfanilyl Chloride-^{35}S (III). Finely-ground phosphorus pentachloride (6.66 g, 31.9 mmole) was added to 3 g (17 mmole) of (II) with ice-cooling, and the mixture triturated. When the reaction was complete, the mixture was heated carefully to give a homogeneous liquid. The liquid was cooled in an ice-bath, and 10 g of crushed ice added to give (III). Yield 2.8 g (89%), mp 147-149°C (from benzene).

N^4-Acetyl-N^1-2-pyrimidinylsulfanilamide-^{35}S (IV). To a cooled suspension of 1 g (10.6 mmole) of 2-aminopyrimidine in 5 ml of dry pyridine was added with stirring 2.8 g (12.7 mmole) of (III). The mixture was stirred for 2 h at 55-60°C, cooled, and 10 g of ice added. The solid which separated was filtered off to give 1.38 g (46.5%) of (IV).

N^1-2-Pyrimidinylsulfanilamide-^{35}S (VI) (sulfadiazine-^{35}S). A solution of 1.38 g (4.73 mmole) of (IV) in 5 ml of 10% NaOH was boiled for 1.5 h. The solution was cooled, diluted with 20 ml of cold water, filtered, and neutralized with dilute hydrochloric acid. The colorless solid was filtered off, washed with water, and dried to give 0.935 g (80%) of (VI), mp 248-249°C (from 60% acetic acid).

EXPERIMENTAL (BIOLOGY)

Method of Tumor Transplantation and Administration of the Drug. Under pentobarbital narcosis, 0.1 ml of a suspension of Brown-Pearce carcinoma tumor cells was introduced via a 2 cm skin incision over the popliteal lymph node of the right rear extremity of the rabbits, directly into the lymph node. After 10-12 days, this node measured 10 × 15 mm and weighed 1-2 g, the values in intact animals being 0.5 × 0.8 mm and 0.2-0.4 g. Also under pentobarbital narcosis, Evans' blue was introduced into the interdigital pulvillus. The stained lymph vessel was punctured with a No. 0425 needle by the modified method described in [1], and 1 ml of a suspension of sulfadiazine-^{35}S (0.8-0.9 MBq/ml) was added over 1-2 min. The weight of drug was 10 mg, and following administration the system was washed through with 0.5 ml of colored physiological saline.

Radiometry of Organs and Tissues. Three or four animals were taken each time the drug was administered. Two, 24, and 120 h following administration, the animals were killed and bioprobes of the organs and tissues obtained. Samples of the organs and tissues weighing 300 mg were hydrolyzed with 2 ml of 1 N caustic alkali on the water bath at 80-90°C for 20 min. To measure the radioactivity, 10 ml of scintillation fluid was placed in a flask, followed by 0.1 ml of the sample hydrolyzate. Radiometry was carried out on an Intertechnique automatic scintillation counter (France). To calculate the levels of radioactivity in the organs and tissues, the radiometry data were expressed as percentages per 1 g organ or tissue weight relative to the activity introduced, taken as 100%.

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