3-[^{11}C]-METHYL-D-GLUCOSE, A POTENTIAL AGENT FOR REGIONAL CEREBRAL GLUCOSE UTILIZATION STUDIES: SYNTHESIS, CHROMATOGRAPHY AND TISSUE DISTRIBUTION IN MICE

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SUMMARY

3-[^{11}C]-Methyl-D-glucose was synthetized by methylation via $^{11}CH_3I$ of the potassium salt of diacetone-D-glucose; hydrolysis of the ketal groups with HCl yields 3-[^{11}C]-methyl-D-glucose. The radiochemical yield is 35% at a specific activity of 1.9 mCi/μmole. Synthesis time including purification by hplc is about 30 min. Up to 12.0 mCi have been obtained in injectable solution.
The tissue distribution of 3-[^{11}C]-methyl-D-glucose was determined in mice at different times after an i.v. injection. All well-perfused organs like lung, heart, liver, kidney and brain rapidly accumulate 3-[^{11}C]-methyl-D-glucose. The accumulation in brain is significant: at 15 min after application it reaches 6.2% dose/g organ.

Key words: Carbon-11, Methyl-D-glucose, Tissue distribution

INTRODUCTION

The normal brain meets its energy needs solely by oxidative metabolism of D-glucose. Therefore, it is obvious to look for potential agents for regional cerebral metabolism studies among D-glucose and its analogues; furthermore, for studies of regional in-vivo metabolism in three dimensions the use of positron emission tomography is indicated. A significant number of different compounds

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that fulfill these requirements have been investigated up to now. Thus, Raichle et al. (1,2) studied the distribution of photosynthetically prepared [^{11}C]-D-glucose in monkey. The multiple pathways of glucose metabolism made it very difficult to interpret the data accumulated during the in-vivo measurements. Ido et al. (3) synthetized the D-glucose analogue [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG). The biodistribution of FDG has been studied by Gallagher et al. (4) in mice, and was successfully applied by Reivich et al. (5) and Phelps et al. (6) to measurements of the regional metabolic rate of glucose in man. FDG is trapped inside the brain cells by the hexokinase reaction to yield ^{18}F-FDG-6-phosphate, which cannot be further utilized for glycolysis. This is an in-vivo application of the well-known autoradiographic [^{14}C]-2-deoxy-D-glucose method described by Sokoloff et al. (7). Using one of these methods (3-7) an integral value of regional cerebral glucose utilization is obtained for the time period between application of the radiopharmaceutical and recording of data. Other compounds have been synthetized using the same approach, like [^{18}F]-3-fluoro-3-deoxy-D-glucose (8) and [^{11}C]-2-deoxy-D-glucose (9,10), but to our knowledge the biodistribution of these two compounds has not yet been extensively investigated.

We felt that it would be useful to investigate a D-glucose analogue which makes differential measurements of changes in regional cerebral glucose utilization possible. Thus, a tracer is desirable which is not metabolically trapped inside the cell.

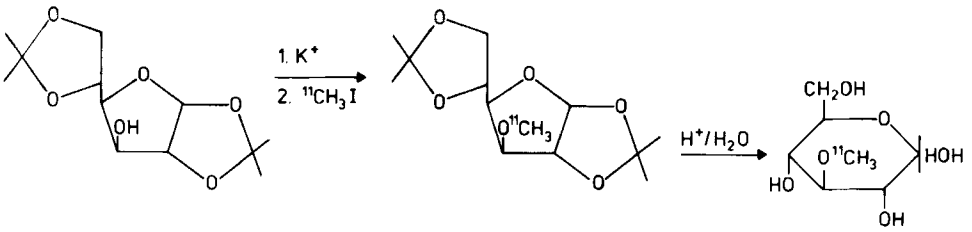
3-Methyl-D-glucose (MG) is transported from blood into brain by the same carrier as D-glucose; the K_M's for both compounds are nearly identical (11). Contrary to the D-glucose analogues mentioned above, MG is not at all metabolized by the body, but is excreted unchanged via the kidneys (12). These useful characteristics should

allow dynamic in-vivo measurements of regional cerebral glucose utilization with ^{11}C-labelled MG, provided that a) glucose metabolism rate is limited by membrane transport, and b) membrane transport of MG is proportional to membrane transport of glucose.

We decided to label MG in the 3-methyl group, since this position is easily labelled via $^{11}CH_3I$ starting from diacetone-D-glucose, in which all other hydroxyl functions are protected.

EXPERIMENTAL

Diacetone-D-glucose was obtained from Fluka AG, Buchs, Switzerland. Practically carrier-free $^{11}CH_3I$ was prepared according to Marazano et al (13).



A typical preparation of 3-[^{11}C]-methyl-D-glucose runs as follows:

To a solution of 100 mg diacetone-D-glucose in 4 ml of diethylether (absolute), 100 mg of potassium are added. The mixture is heated under reflux for 30 min. The reaction mixture is left for one to three days at room temperature in order to achieve a more complete product formation.

t=0 min Practically carrier-free $^{11}CH_3I$ (78 mCi) was transferred into 0.7 ml of this solution with a stream of helium gas. The resulting mixture was transferred into a 2 ml ampoule, sealed with a teflon septum and heated at 120 ^{o}C for 6 min. After this time, the diethylether was evaporated.

t=7 min To the residue 0.6 ml 1 N HCl and 0.6 ml water : methanol 1 : 1 were added with a syringe; the resulting solution was heated at 120 °C for 6 min.

t=14 min The ampoule was opened, the mixture transferred to a 25 ml flask and evaporated to dryness using a rotary evaporator.

t=17 min The residue was taken up in 1 ml acetonitrile : water 4 : 1 and injected onto a hplc-column (LiChroSorb-NH_2, 10 µ, 25 x 1 cm) via a sample valve. At a flow rate of 2 ml/min using acetonitrile : water 4 : 1 as eluent, [^{11}C]-MG was eluted with a retention time of 3.5 min.

t=24 min The [^{11}C]-MG was collected and evaporated to dryness

t=27 min using a rotary evaporator.

An optimum radiochemical yield of 44.7% ≙ 13.9 mCi (decay corrected) was obtained at a specific activity of 1.9 mCi/µmole. The yield was calculated from 19 experiments to be 35 ± 3% with a mean preparation time (including chromatography) of 34 min.

During preparation of $^{11}CH_3I$, about 0.3 - 1.0 µmole of non-radioactive CH_3I are generated, probably from atmospheric CO_2; therefore, MG is not carrier-free. Addition of up to 70 µmole of carrier CH_3I does not change the yields within the relatively large experimental error. The hplc conditions (Table I) yield a salt-free residue of 3-[^{11}C]-MG; thus, no problems with the osmolarity of the injection solution are encountered.

Table I. Chromatographic data*

Compound	k'
3-[^{11}C]-methyl-D-glucose	1.0
D-glucose	2.4

*Column: µ-Bondapak-NH_2, 10 µ, 30 x 0.4 cm
eluent: acetonitrile : water 4 : 1
flow: 2 ml/min

For administration to mice, 3-[^{11}C]-MG was taken up in 1-10 ml isotonic saline and filtered through a 0.2 µm Millipore filter to yield a sterile solution suitable for injection. Up to now, a maximum of 12 mCi of 3-[^{11}C]-MG was obtained in injectable solution. By application of automatic synthesis procedures it will be possible to prepare significantly larger amounts of 3-[^{11}C]-MG.

ANIMAL EXPERIMENTS

Throughout the study female NMRI albino mice with body weights ranging from 27-33 g were used. 1-10 µCi of 3-[^{11}C]-MG in 100 µl isotonic saline were injected into the tail vein of the animals confined to a restriction cage. Animals were killed by cervical dislocation after the appropriate time intervals. The organs of interest were removed, blotted dry, counted in a well-type counter using a NaI(Tl)-scintillation detector, and weighed.

Since there was a rather large variation in body weight of the animals, the results are expressed as % mean body concentration (% MBC) in various organs; the % MBC is obtained by dividing specific activity of the organs (cpm/g) by the applied dose (cpm/g body weight) and multiplying by 100.

DISCUSSION

All well-perfused organs like lung, heart, liver, kidney and brain rapidly accumulate 3-[^{11}C]-MG after an i.v. injection (see Table II).

The accumulation of 3-[^{11}C]-MG by the brain is significant for the time interval between application and 15 min after application; at 15 min the concentration of radioactivity in the brain is 175% MBC $\hat{=}$ 6.2% dose/g organ. The brain-to-blood ratio rises to about 0.7 during the same time interval. This compares favorably with data obtained with [^{3}H]-MG in mice by Wassenaar et al. (14).

Table II. Tissue Distribution of 3-[^{11}C]-methyl-D-glucose at Different Times after I.V. Injection (n = Number of Animals)

		Organ investigated								
Time (min)		brain	blood	kidney	liver	intestine	muscle	uterus	heart	lung
0.16	(n=7)	146±46	389± 82	302±150	192± 85	86±40	107±30	105±85	296± 91	269± 57
0.25	(n=6)	70±23	247±103	294±196	168± 35	81±19	n.d.	90±83	257±134	213± 66
0.5	(n=4)	95±37	418± 49	727± 63	240± 7	112±10	80±10	77± 8	196± 18	304± 89
1.0	(n=8)	129±54	403± 75	895±410	298±117	149±67	117±40	162±76	266±116	287±150
1.25	(n=8)	143±11	466±110	1041±458	322± 39	159±27	114±30	184±76	275± 43	292± 71
1.5	(n=4)	112±21	264± 42	628± 14	224± 26	105±13	64± 7	100±19	176± 36	151± 43
1.75	(n=4)	151±28	271± 46	598± 59	253± 28	119±16	76±14	120±59	165± 14	176± 26
2.0	(n=6)	123±48	230± 99	513±251	232± 85	97±33	83±46	130±31	202±111	165± 77
2.5	(n=5)	106±13	224± 5	525± 70	193± 20	101± 7	68±14	138±31	145± 25	167± 19
3.0	(n=4)	163±18	311± 26	703± 90	296± 27	133±16	106±32	150±11	241± 30	214± 31
10.0	(n=5)	133±21	285± 46	662±237	227± 21	159±58	88±15	224±61	180± 37	226± 25
15.0	(n=3)	175±70	268± 8	421± 61	201± 12	115± 9	132±34	186±37	204± 7	208± 18
30.0	(n=4)	45±15	106± 29	202± 68	82± 27	57±17	42± 5	75±40	76± 25	81± 30
60.0	(n=4)	19± 3	46± 16	149±123	37± 6	33± 4	39±28	48±40	35± 5	43± 6

Values expressed as % MBC (see text)

Values are mean ± S.D.

But it is less than in the case of [^{18}F]-FDG (4), where about 18% dose/g brain have been found. But since we are investigating a tracer that can be freely transported back and forth through the blood-brain barrier (11), this difference is not surprising.

The concentration of radioactivity in the heart muscle is slightly larger than that in the brain. As in the case of [^{18}F]-FDG (15), 3-[^{11}C]-MG also seems to be a promising agent for studies of the myocardial glucose utilization (16).

As expected, the concentration of radioactivity in the kidneys, the main route of excretion (12,14), was very high. The excretion is rapid; 60 min after application only the kidneys retain radioactivity slightly above 100% MBC. All other organs studied contain only very little radioactivity.

Due to the high heart rate in mice, the maximum accumulation of radioactivity in various organs is expected to occur very early after i.v. injection. Using other animal species or man, the maximum accumulation is expected to occur significantly later due to the lower heart rate. In mice we observed a broad maximum of accumulation in the brain between 1 and 15 min after application; thus, we expect the maximum between 1 min and 1 hour after application in larger animals or in man. Because of the radioactive decay it is difficult to collect data for more than 1 hour after application with a carbon-11 labelled compound.

Thus, we feel that 3-[^{11}C]-MG is a useful radiopharmaceutical for the study of regional cerebral glucose utilization. Investigations in larger animals and in man are presently in progress.

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