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Original Paper

The Effect of Different Dose Levels of Degradable Starch Microspheres (Spherex®) on the Distribution of a Cytotoxic Drug After Regional Administration to Tumour-bearing Rats

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Tumour uptake of a radiolabelled anticancer drug, tauromustine ([^{14}C]TCNU), was investigated, using two different routes of administration in rats with colon adenocarcinomas implanted in the liver. Intra-arterial administration produced higher concentrations of the drug and/or its metabolites in tumour tissue and lower concentrations in normal tissue compared to intravenous administration. The investigation of co-injection of [^{14}C]TCNU intra-arterially with degradable starch microspheres (DSM) indicated that a high dose of DSM (30 mg/kg) resulted in a high concentration of radioactivity in normal liver tissue, and in adjacent organs. This unfavourable pattern was not observed with the low dose of DSM (12.5 mg/kg), which produced a significant increase in tumour uptake. The results demonstrate the efficacy of partial vascular blockade, as elicited by the low dose of DSM. This regime caused [^{14}C]TCNU to be preferentially retained in the active peripheral regions of the tumour.

Key words: degradable starch microsphere, Spherex®, TCNU, intra-arterial, distribution, tumour, experimental
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INTRODUCTION

THE EFFECTIVENESS of intra-arterial chemotherapy is enhanced by combination with occlusive agents [1]. Degradable starch microspheres (Spherex®, DSM) can be used repeatedly to produce transient vascular occlusion for approximately 1 h [1]. DSM has been used both experimentally and clinically in the treatment of liver cancer, with the aim of increasing the extraction and efficacy of different cytotoxic drugs [1]. However, it has been noted that local toxicity may become a dose-limiting factor after occlusion [2].

Nitrosoureas are a group of anticancer drugs, characterised by a high clearance in the vascular compartment, indicating their potential as suitable drugs for regional use in combination with DSM [3]. The clinical efficacy of nitrosoureas has been documented [4, 5]. In previous experiments in rats with solitary liver tumours, co-administration of DSM increased the antitumour effect of tauromustine (TCNU). However, local toxicity was also increased, resulting in liver damage [6, 7]. Therefore, it is necessary to perform dose-finding studies to determine the optimum dosing regimen for DSM, using a suitable animal model. The aims of this study were to determine the distribution and time dependency for tumour uptake of intravenously administered [^{14}C]TCNU, and to compare the tumour uptake of this cytotoxic drug, after intra-arterial co-injection, with different dose levels of DSM.

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MATERIALS AND METHODS

Test compound and dosage

TCNU (1-(2-chloroethyl)-3-[2-(dimethyl-aminosulphonyl)-ethyl]1-nitrosourea; Pharmacia AB, Sweden) was dissolved in saline immediately before use. [^{14}C]TCNU, with a specific activity of 36.1 μCi/mg and a radiochemical purity of 95%, was synthesised at Pharmacia, Sweden. DSM (Spherex®, Pharmacia, Sweden), with a mean diameter of 45 μm, was administered as a suspension containing either 60 mg/ml or diluted with saline to a final concentration of 25 mg/ml.

Animals and tumours

Thirty-five inbred male Wistar SU rats (Alab, Sollentuna, Sweden) weighing approximately 200 g were used in the study. The experimental tumour was a dimethylhydracin-induced

adenocarcinoma of rat colon, propagated by intraperitoneal transplantation in rats [8].

Study design

A suspension of 10^6 viable tumour cells in 0.1 ml of phosphate-buffered saline was injected under the capsule of the left lateral liver lobe [9]. After 10 days, a catheter (PE 10, Portex, Hythe, Kent, U.K.) with an inner and outer diameter of 0.28 mm and 0.61 mm, respectively, was inserted retrogressively into the gastroduodenal artery with the tip close to the origin of the proper hepatic artery. Retrograde flow into the common hepatic artery during the injection was prevented by placing a loop around the vessel.

Twenty-seven animals received a bolus injection of TCNU (1.5 mg/kg, 54 μCi/kg) through the catheter, combined with 0 (eight animals), 12.5 (10 animals) or 30 (nine animals) mg/kg DSM. The dose was given in a volume of 1.8 ml/kg and injected over 90 s. In a separate experiment, using eight animals, the same dose of TCNU was injected intravenously into a tail vein over 90 s.

Anaesthesia

Surgery was performed under nitrous oxide–isoflurone anaesthesia: 4% isofluorone in a gaseous mixture of 1 l oxygen and 2 l nitrous oxide (N_2O) per minute was used for 3 min for induction, and then animals were maintained on 1.5% isoflurone [10].

The whole body autoradiography procedure

The technique of whole body autoradiography (WBA) was performed according to the method described by Ullberg [9]. In brief, animals were killed by CO_2 inhalation at 20 min, 2 and 8 h after intra-arterial administration, and 5 min, 20 min, 1, 2, 4, 8, 24 and 48 h after intravenous administration. The animals were then mounted for sectioning in a gel of carboxymethyl cellulose (CMC), and rapidly frozen in hexane cooled with dry ice (−78°C). From each rat, 20 μm thick sagittal sections were cut at different levels with a cryomicrotome at a temperature of −20°C. The resulting sections were caught on adhesive tape. After being freeze-dried at −20°C for at least 24 h, the sections containing the ^{14}C-radioactivity were exposed to an X-ray film (Structurix Agfa, Gaevert). After an exposure period of approximately 4 weeks, sections and films were developed under standardised conditions, fixed, rinsed and dried. The resulting autoradiograms were then analysed by computerised image analysis [11], and the densitometric readings obtained from the autoradiograms were converted to radioactivity values using radio standards (Micro-scales, Amersham International Ltd., U.K.). Results from the following organs were quantified; muscle, bone marrow, thymus, lung, spleen, pancreas, stomach, liver lobes (ipsilaterally and contralaterally to the tumour) and tumour.

Calculations and statistical analysis

The mean tissue concentration of the drug was calculated from different animals and expressed as nCi/mg of tissue. The "target index" was defined as the ratio of the high radioactivity zones in tumour tissue and those in normal liver tissue (contralaterally to the tumour). Statistical analysis was performed using Students *t*-test. P values of < 0.05 were considered to be statistically significant.

RESULTS

Tables 1 and 2 show the tissue concentrations of radioactivity after intravenous and intra-arterial administration of [^{14}C]TCNU alone or in combination with either high dose (30 mg/kg) or low dose (12.5 mg/kg) DSM. The tissue radioactivity, expressed as the mean nCi/g, is shown for relevant organs; for the other organs examined, no differences between treatments were found (data not shown).

The autoradiograms obtained 20 min and 2 h postadministration are presented in Figures 1 and 2, respectively. Within the tumour tissue, certain areas of high radioactivity were noticed (Figure 2d). The uptake in these high radioactivity zones was generally approximately twice that of the mean tumour uptake. The calculated target index for each of the treatments is shown in Figure 3.

Distribution of [^{14}C]TCNU after intravenous administration

There was a rapid distribution of radioactivity within the body after intravenous administration of [^{14}C]TCNU. As early as 20 min after administration, the extensive distribution gave rise to maximum concentrations in most tissues examined (Table 1). However, the radioactivity was not evenly distributed. The radiolabelling of the lung was similar to that of blood, but levels higher than those found in blood were initially measured in the liver (data not shown).

Tumour tissue initially showed a lower mean concentration of radioactivity, as compared to normal liver tissue. There was also a more rapid elimination of [^{14}C]TCNU from normal liver tissue compared to tumour tissue. For the high radioactivity zone of the tumour, the target index slowly increased over time and a maximum value of 1.5 was achieved 8 h after the injection.

Persistently high levels of radioactivity were also found in some other tissues. Thus, only at the later time points, 2–8 h postadministration, did the levels in the exocrine pancreas and thymus reach a maximum.

Distribution of [^{14}C]TCNU after intra-arterial administration

Twenty minutes after intra-arterial administration, the concentration of radioactivity in tumour tissues was higher than in the normal liver (Table 2), resulting in a high target index of 4.0 ± 3.1 (mean ± S.D.) (Figure 3). With the exception of this first time point, a similar pattern of distribution in the tumour area was found irrespective of the route of administration (Table 2). Furthermore, as compared to intravenous administration, lower levels of radioactivity were observed outside the tumour areas following intra-arterial administration.

Distribution of [^{14}C]TCNU combined with a high dose of DSM

The distribution of ^{14}C-labelled TCNU co-injected with a high dose of DSM, 30 mg/kg, is illustrated in Figure 2c. Using this high dose of DSM, the mean tumour concentration of radioactivity (nCi/g) was not significantly different from that obtained after intra-arterial treatment without DSM and there was a large variation between animals seen early after administration (20 min); the mean values (± S.D.) were 62 (± 54) and 190 (± 170), respectively (Table 2). However, the tendency for lower tumour levels in the DSM group resulted in a significant difference between groups in the last measurement performed at 8 h, mean tumour levels were 14 (± 7) and 31 (± 3) in the group with and without DSM, respectively (Table 2). A similar trend was also evident for the target index (Figure 3).

Locally, in the surrounding normal liver tissue, as well as in adjacent organs, higher ^{14}C-concentrations were observed in the DSM group, with a significant difference being found 20 min postadministration in the normal liver and the stomach. In peripheral tissues, such as muscle, similar concentrations of

Table 1. Tissue concentration of radioactivity after intravenous injection of ^{14}C-labelled TCNU, 1 animal per time point

Tissue	Time 5 min	20 min	60 min	2 h	4 h	8 h	24 h	48 h
Tumour (high radioactivity zone)	60 ± 1	83 ± 5	57 ± 11	69 ± 2	57 ± 0.4	41 ± 2	12 ± 1	9 ± 1
Tumour	38 ± 2	50 ± 3	26 ± 10	32 ± 6	28 ± 6	31 ± 1	7 ± 1	6 ± 1
Liver ipsilateral	98 ± 1	142 ± 0.3	85 ± 7	78 ± 9	55 ± 4	31 ± 2	10 ± 1	9 ± 1
Liver contralateral	111 ± 0.4	145 ± 8	87 ± 16	87 ± 16	53 ± 3	28 ± 1	10 ± 1	9 ± 1
Lung	63 ± 1	100 ± 10	41 ± 2	47 ± 2	20 ± 4	21 ± 1	8 ± 1	5 ± 1
Muscle	40	44	20	21	1	8	3	3
Thymus	38 ± 5	48 ± 2	19 ± 1	47 ± 2	56 ± 6	63 ± 1	32 ± 1	16 ± 1
Spleen	59 ± 3	72	37	46 ± 10	32	20	6	6
Pancreas	66 ± 0.3	72 ± 0.3	59	85 ± 12	82 ± 15	59 ± 4	15 ± 2	8 ± 0.3
Stomach	41 ± 3	52 ± 15	34	39 ± 12	30 ± 12	20 ± 1	8	4 ± 0.3
Bone marrow	38 ± 1	39	35	38	8	15	6	4

The optical densities obtained from whole body autoradiograms were converted to concentration units (nCi/g; mean ± S.D.). The S.D. is the variation for different measurements within each tissue.

Table 2. Tissue concentration of radioactivity after intra-arterial (i.a.) administration of ^{14}C-labelled TCNU with and without DSM (30 or 12.5 mg/kg)

Tissue	20 min i.a.	20 min i.a. + 30 mg/kg DSM	20 min i.a. + 12.5 mg/kg DSM	2 h i.a.	2 h i.a. + 30 mg/kg DSM	2 h i.a. + 12.5 mg/kg DSM	8 h i.a.	8 h i.a. + 30 mg/kg DSM	8 h i.a. + 12.5 mg/kg DSM
Tumour (high radioactivity zone)	259 ± 195	122 ± 70	486 ± 259	30 ± 3	74 ± 38	167 ± 59*	50 ± 3	22 ± 7*	49 ± 6
Tumour	190 ± 170	62 ± 54	338 ± 140	20 ± 8	50 ± 30	125 ± 63*	31 ± 3	14 ± 7*	29 ± 4
Liver ipsilateral	71 ± 6	118 ± 24*	110 ± 47	62 ± 11	82 ± 22*	82 ± 23	37 ± 4	41 ± 7	28 ± 6
Liver contralateral	67 ± 5	86 ± 10*	99 ± 31	63 ± 13	81 ± 22	77 ± 19	38 ± 1	43 ± 6	29 ± 4
Lung	38 ± 13	37 ± 11	48 ± 13	17 ± 7	25 ± 6	22 ± 17	7 ± 1	11 ± 6	5 ± 2
Spleen	27 ± 5	95 ± 129	49 ± 8*	25 ± 7	23 ± 8	34 ± 0	13 ± 2	16 ± 9	9 ± 3
Pancreas	35 ± 6	141 ± 172	47 ± 19	42 ± 8	53 ± 20	45 ± 23	42 ± 1	59 ± 14	36 ± 6
Stomach	35 ± 2	114 ± 102	56 ± 12*	35 ± 15	33 ± 10	20 ± 18	15 ± 8	11 ± 8	10 ± 3

The optical densities obtained from the whole body autoradiograms were converted to concentration units (nCi/g; mean ± S.D.). S.D. is the variation between different animals.
*Significantly different from vehicle-treated control (intra-arterial) $P < 0.05$.

radioactivity were found irrespective of the addition of DSM to the intra-arterial treatment (data not shown).

Distribution of [^{14}C]TCNU combined with a low dose of DSM

The distribution of ^{14}C-labelled TCNU co-injected with a low dosage of DSM, 12.5 mg/kg, was notably different from that with a high DSM dose, as illustrated in Figure 2d. Increased tumour drug levels were found within the first 2 h following intra-arterial administration of [^{14}C]TCNU combined with 12.5 mg/kg DSM, as compared with the intra-arterial control group which received [^{14}C]TCNU alone. The mean tumour concentrations (nCi/g) were 338 (± 140) and 190 (± 170) at 20 min and 125 (± 63) and 20 (± 8) at 2 h ($P < 0.05$) in the intra-arterial group with or without DSM, respectively, whereas similar values were found after 8 h (Table 2). The high concentrations of radioactivity found in tumour tissue within 2 h postadministration were also reflected in a high target index, which was significantly different from that of the vehicle-treated control group (Figure 3).

A DSM-induced increase in radioactivity of adjacent regional organs was only found immediately after administration. Thus at 20 min after administration of the low dose DSM regimen, significantly higher levels of radioactivity were found in the spleen and stomach as compared with data obtained in the control group with intra-arterial [^{14}C]TCNU alone (Table 2). No significant differences were found in peripheral tissues, such as muscle, in terms of the effect of adding 12.5 mg/kg DSM to the intra-arterial regimen (data not shown).

DISCUSSION

In this study, the distribution of a radiolabelled cytotoxic drug, TCNU, was examined in tumour-bearing rats using the technique of whole body autoradiography. The advantages of using quantitative autoradiography are that the investigation is not limited to certain preselected tissues. Thus, it permits unforeseen localisations and heterogeneic tissue uptakes to be detected and quantified. Firstly, the distribution of TCNU was studied over 2 days following intravenous administration. Results showed that radioactivity was rapidly distributed, and peak values in tissues were observed within the first hour after administration, although no preferential tumour uptake was seen. However, the distribution within the tumour was not

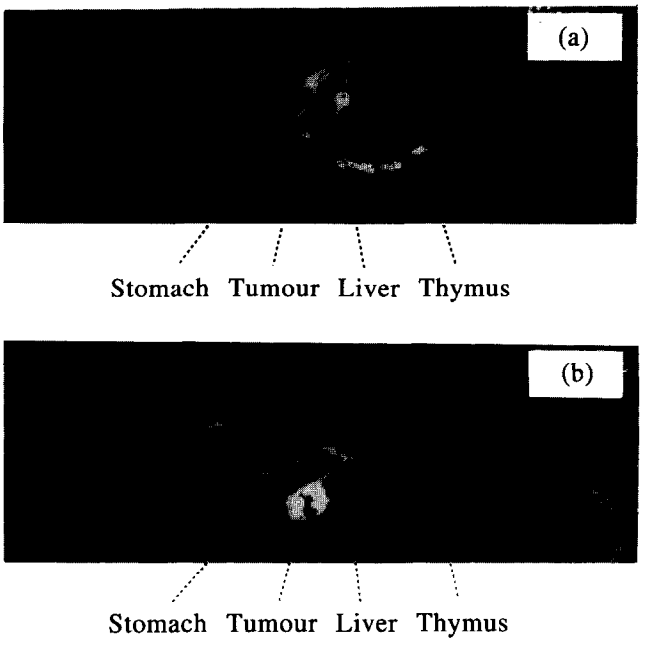

Figure 1. The distribution of [^{14}C]TCNU at 20 min (a) after intra-arterial administration combined with a high dose of DSM; and (b) after intra-arterial administration combined with a low dose of DSM. Note the high concentrations of radioactivity in the tumour after intra-arterial administration with a low dose of DSM. When a high dose of DSM was used, a very high concentration of radioactivity was obtained within the liver, and radioactivity also spread into other locally adjacent tissues.

uniform. Higher concentrations occurred around the active peripheral regions of the tumours as compared to central areas, probably reflecting a high degree of necrosis in the central region. This is supported by a recent study [6] using the same experimental model, where the effect of a cytotoxic drug (doxorubicin) alone or combined with DSM was compared [6]. Histological examination showed necrotic areas in the liver and tumour of rats treated with the DSM combination. A thin layer of surviving cells was found on the tumour periphery [6].

Over time, we found a persistence of radioactivity within the tumour, while it was more rapidly eliminated from normal tissues. Thus, when comparing concentrations of the radioactivity within the active tumour to that of normal liver tissue, a maximum target index of approximately 1.5 was found 8 h after intravenous administration.

Results from intra-arterial administration of TCNU showed rapid tumour uptake, resulting in a high target index 20 min after administration. This preferential tumour uptake indicates a hypervascular tumour and an experimental model suitable for analysing the effect of intra-arterial regimens. Furthermore, lower concentrations of radioactivity in peripheral organs indicated lower systemic exposure due to increased extraction of drug during the first passage in the tumour area. Obviously, the increased regional drug concentration in the tumour area was only transient and was followed by a wash-out phase of the radiolabelled drug. Thus, a targeting effect was found only up to 20 min after intra-arterial administration of [^{14}C]TCNU.

The main objective of this study was to optimise the local distribution of TCNU by concomitant injection with DSM. Therefore, [^{14}C]TCNU was given either together with a relatively high (30 mg/kg) or a relatively low (12.5 mg/kg) dose of DSM. The high dose of DSM has previously been investigated

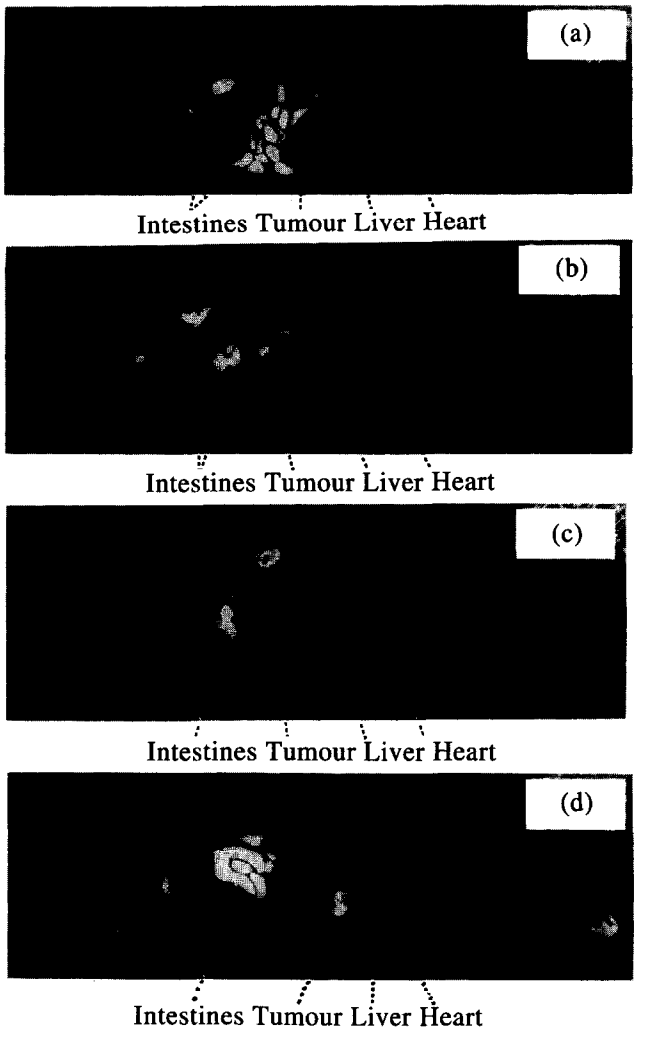

Figure 2. The distribution of [^{14}C]TCNU at 2 h (a) after intravenous administration; (b) after intra-arterial administration; (c) after intra-arterial administration combined with a high dose of DSM; and (d) after intra-arterial administration combined with a low dose of DSM. Note the low concentrations of radioactivity in the tumour after intravenous as well as after intra-arterial administration and the high concentration of radioactivity in the tumour with a low dose of DSM.

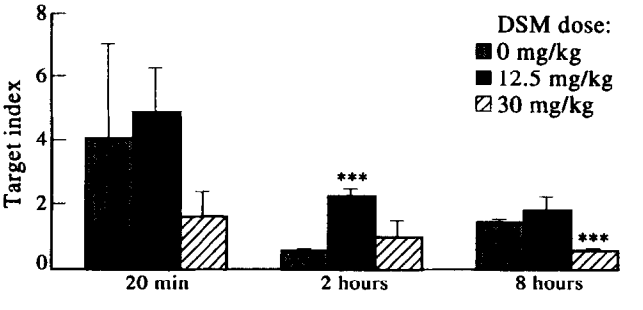

Figure 3. Target index (mean ± S.D.) calculated as the ratio of ^{14}C-radioactivity between high radioactivity zones within tumour tissue and contralateral liver lobe following intra-arterial administration. * significantly different from vehicle treated control (intra-arterial), $P < 0.001$.**

in pharmacotoxicological studies, and, although shown to be effective, increased regional toxicities, such as liver and gastric necrosis, were observed [6, 7, 12]. These negative effects of large amounts of DSM may be explained by a total block of the hepatic artery, forcing the spheres to enter retrogressively into the splanchnic circulation where they may temporarily occlude splanchnic organs. A similar effect is clearly illustrated in the present study in animals that were administered 30 mg/kg DSM. The drug concentration increased 3–4-fold in spleen, stomach and pancreas after this high DSM dose. Such an indication of backflow during drug injection confirms the necessity of recording the pressure during injection in the animal model [13] when giving high doses of DSM.

In contrast, when TCNU was given intra-arterially together with a low dose of DSM, a quite different distribution profile was found. Immediately after the low dose of DSM, the concentration of radioactivity was only slightly increased in adjacent organs. Furthermore, the most interesting finding of this study is that a tumour-targeting effect was evident when co-injecting TCNU with a low dose of DSM, i.e. 12.5 mg/kg. A 5-fold increase of radioactivity was observed 2 h postadministration. At this timepoint, 2 h after DSM injection, vessels are no longer occluded [1]. Thus, TCNU should not be trapped within vessels, but should be distributed into the tissues.

The results of the present study may be explained by the immature regulation of tumour blood flow [14]. It can be estimated that most tumour vessels are permanently open [14, 15], whereas only a minor fraction of the normal liver vessels are open simultaneously. New vascular areas have been shown to open up when hypoxia is induced by injection of microspheres [15, 16]. Based on these considerations a higher proportion of the microsphere and co-administered drug will be available in the tumour area during the initial phase of treatment. In contrast, with a higher dose of DSM producing almost complete arterial occlusion, the blood flow through the tumour might be blocked and more of the drug becomes available to the normal liver.

When used clinically, the optimal DSM dose per patient is established prior to cytotoxic drug administration [15–17]. Using such a treatment approach, the normal liver parenchyma might receive prolonged exposure to the co-administered cytotoxic drug. However, according to the results achieved in our experimental model, a suboptimal dose of DSM can give preferential blocking in the tumour area. Thus, when using DSM in intra-arterial liver tumour treatment, the highest regional advantage should be achieved by a dose of DSM producing a partial vascular blockade.

In conclusion, in this study, we have demonstrated the advantage of a partial, as opposed to a total, vascular blockade with the use of low dose DSM. With the low dose of DSM used in this study, the co-injected drug was shown to be preferentially retained in the active peripheral regions of the tumour.

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